Endocrine toxicity of fluoroquinolones

Investigations on their insulinotropic effect

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina

zu Braunschweig

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

genehmigte

D i s s e r t a t i o n

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eingereicht am: 26.07.2010

mündliche Prüfung (Disputation) am: 30.09.2010

Druckjahr 2010

Vorveröffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Fakulät für Lebenswissenschaften, vertreten durch den Mentor der Arbeit, in folgenden Beiträgen vorab veröffentlicht

Publikationen

1- Ghaly H, Kriete C, Sahin S, Pfloeger A, Holzgrabe U, Zünkler BJ, Rustenbeck I (2009): The insulinotropic effect of fluoroquinolones. Biochem Pharmacol; 77:1040-1052.

2- Willenborg M, Ghaly H, Hatlapatka K, Urban K, Panten U, Rustenbeck I (2010): The signalling role of action potential depolarization in insulin secretion: metabolism-dependent dissociation between action potential increase and secretion increase by TEA. Biochem Pharmacol; 80(1):104-112.

3- Willenborg M, Hatlapatka K, Ghaly H, Belz M, Panten U, Rustenbeck I (2010): Studies of first phase insulin secretion using imposed plasma membrane depolarization. Frontiers in Bioscience (accepted for publication).

Tagungsbeiträge

1- Ghaly H, Kriete C, Sahin S, Pfloeger A, Holzgrabe U, Zünkler BJ, Rustenbeck I (2009): The insulinotropic effect of fluoroquinolones. Nauny-Schmiedeberg's Archives of Pharmacology 379 (Suppl 1):163.

2- Ghaly H, Sahin S, Zünkler BJ, Rustenbeck I (2009): Effects of fluoroquinolones on mitochondrial energetics in mouse pancreatic islets and islet cells. Islet Study Group Symposium, Vienna, Austria October 2-4.

3- Ghaly H, Hatlapatka K, Rustenbeck I (2010): The effect of fluoroquinolones on pancreatic B-cell energy metabolism. Nauny-Schmiedeberg's Archives of Pharmacology 381 (Suppl 1):148.

4- Ghaly H, Hatlapatka K, Rustenbeck I (2010): The insulinotropic effect of fluoroquinolones is counteracted by their inhibitory effect on pancreatic B-cell energy metabolism. Diabetes und Stoffwechsel; 5: 316.

5-Willenborg M, Ghaly H, Belz M, Panten U, Rustenbeck I (2010): Das Aktionspotential und die Insulinsekretion – ein zwingender Zusammenhang? Diabetes und Stoffwechsel; 5: 81.

ACKNOWLEDGMENT

I would express my most grateful acknowledgements to:

First of all to **GOD** for his constant presence in my life, administering my plans and guiding my steps; for his light in times of darkness; for his comfort in times of grief and for the opportunity of life.

To Prof. Dr. Ingo Rustenbeck for encouraging me to perform my PhD Study in Germany and for introducing me to the fascinating field of pancreatic islets research. His enthusiasm for science and never-ending supply of insightful ideas has been a continual inspiration to me. I am also thankful to him for many chances to present parts of my work at international conferences. I appreciate the efforts done by Prof. Anne Jörns for the possibility to perform electron microscopic studies in Medical School of Hannover.

I would also like to thank all technical assistants in the lab, especially Verena Lier-Glaubitz for her unrestricted help especially in the first months of my work. I enjoyed the time working with her. I am sincerely thankful to Angela Hahlbohm for the ATP /ADP measurements. Additionally, I would like to express my gratitude to Sabine Warmbold and Carolin Rattunde.

I am grateful to my colleagues; Michael Willenborg, Michael Belz, and Dr.Kathrin Hatlapatka for the wonderful working atmosphere, the advices as well as for the fruitful discussions.

I am also very grateful to all my colleagues and professors at the faculty of Medicine, Assiut University, Egypt for their continuous support.

To my family, for their support and motivation throughout the project even so far away from me. In special thanks to extremely supportive wife for all the love and warmth she gives to me and for helping me to surpass every difficulty I encounter. Without her, the completion of this project would have been an impossible task. I would also never forget all the efforts done by Prof. Sozan Tous; my mother in law, who spend a lot of time to follow meticulously the administrative steps.

Finally, I thank all who participated in my walk, criticizing me, encouraging me, and helping me.

List of Abbreviation

ADP	Adenosine diphosphate
ADRs	Adverse drug reactions
AM	Acetoxy methyl
ATP	Adenosine-5'-triphosphate
BP	Band pass filter
BSA	Bovine serum albumin
cAMP	Cyclic adenosine-3',5'-monophosphate
$[Ca^{2+}]_i$	Intracellular calcium ion concentration
CBS	Chromatic beam splitter
CCCP	Carbonyl cyanide-m-chlorophenylhydrazone
CCD	Charge coupled devices
CID	Clinical Infectious Diseases
CDAD	Clostridium difficile–associated disease
CNS	Central nervous system
C_{max}	Maximum concentration
CTM-05	Counter/timer board
CYP1A2	Cytochrome P450 1A2
Delta Psi ($\Delta\Psi m$)	Mitochondrial Inner Membrane Potential
D-600	Methoxyverapamile
D.C.	Direct current
DMSO	Dimethyl sulfoxide
ECL	Emitter-Coupled Logic
EGTA	Ethylene glycol tetra-acetic acid
EM	Electron microscope
ER	Endoplasmic reticulum
EU	European Union
F	Fluorescence
$FADH_2$	Reduced flavin-adenine-nucleotide
FCS	Fetal calf serum
FDA	Food and Drug Administration
g	Gram
GFL	Geslleshaft Für Labortechnik
GLUT	Glucose transporter
GSIS	Glucose stimulated insulin secretion
h	Hour
HEK cells	Human Embryonic kidney cells
HEPES	(4-(2-Hydroxyethyl)-1-piperazine ethane sulfonic acid)
HERG	Human ether-a-go-go-related gene
HIT	clonal hamster β cell line
i.e.	id est. (That is to say)
K_{ATP}-channel	ATP-sensitive potassium channel
KR	Krebs-Ringer
L	Litre
LP	Long pass filter
M	mol/l
mm	Millimetre
mg	Milligram
ml	Millilitre
mV	Millivolt
min	Minute

mM	Millimolar (10^{-3}mol/l)
M receptors	Muscarinic receptors
N	Normal
NA	Numerical aperture
NAD(P)H	Nicotinamide-Adenine-Dinucleotide-Phosphate
NMDA	N-methyl-D-aspartate
NMRI	**N**aval **M**edical **R**esearch **I**nstitute
µm	Micrometer
µM	Micromolar (10^{-6}mol/l)
µl	Microliter (10^{-6}l)
n	Number of experiments or islets.
NMRI	Naval Medical Research Institute
PEC	Paul–Ehrlich-Society of Chemotherapy
PK	Pyruvate Kinase
pmol	Picomole (10^{-12} mol)
PP cells	Pancreatic polypeptide cells
QTc	Corrected QT interval
RER	Rough endoplasmic reticulum
RINm5F	Rat Insulinoma cell line
ROI's	Regions of interest
s	Seconds
SEM	Standard error of the mean
SERCA	Sarco-endoplasmic reticulum calcium transport ATPase
SNR	Signal to noise ratio
SUR	Sulfonylurea receptor
Tdp	Torsades de pointes
TTL	Transistor -Transistor Logic
UV	Ultraviolet
VDCC	Voltage-dependent calcium channel

1. Introduction

1.1 Anatomy of the Pancreas

1.1.1 Gross Anatomy of the Pancreas

The pancreas (from the Greek meaning ‘all flesh’) is a retroperitoneal organ located posterior to the stomach on the posterior abdominal wall. Macroscopically, the pancreas in humans is a small elongated well-defined organ of compact structure, whereas in rodents it is distributed between different parts of the intestines and the spleen and not easily distinguishable from the surrounding adipose tissue. The pancreas develops from the innermost of the 3 germ layers called the endoderm. After the gut tube has formed in the embryo, several buds grow below the section that widens to become the stomach. These buds extend and branch to generate the liver, the gallbladder, and the pancreas rudiments (Spooner et al., 1970). Differential rotation and fusion of the ventral and dorsal pancreatic buds results in the formation of the definitive pancreas. The dorsal pancreatic bud gives rise to the anterior part of the head of the pancreas, in addition to the body and tail, while the ventral pancreatic bud develops into the posterior part of the pancreatic head (Slack, 1995). Fusion of the pancreatic buds is accompanied by anastomosis of the ducts. The main drainage duct of the ventral pancreatic bud communicates with the main duct of the dorsal pancreatic bud, with the point of union lying between the isthmus and head of the pancreas. This becomes the dominant and more constant pancreatic duct; duct of Wirsung (Kamisawa, 2004). The cells of the branching pancreatic buds differentiate according to their functions. The cells at the end of the branches form a cap of acinar (Latin: berry) cells, producing digestive juice. The cells lining the branches become ductal cell, transporting the products of the acinar cells to the intestine. By time, a special group of cells detaches from the branches and assembles into small colonies called pancreatic islets. These cells will eventually become the signaling centers of the pancreas (Collombat et al., 2006).

1.1.2 Microscopic Anatomy

The normal adult human pancreas is composed of exocrine tissue (80 % of the total mass), ducts, vessels, nerves, and connective tissue (18 % of the total mass) and endocrine tissue (2 %); about 1 million islets. Islets of Langerhans, named after their discoverer Paul Langerhans, constitute a unique endocrine organ consisting of insulin-producing β cells (65-80 % of the total islet cell population), glucagon-producing α cells (15-20 %), somatostatin-producing δ cells (3-10 %), ghrelin-producing ε-cells, and pancreatic polypeptide (PP) cells (Rahier et al., 1981). Across species, there are major differences in islet structure, distribution of islets into particular parts of the gland, and regional variations in the numbers of specific islet cells. In rodent islets,

the vastly predominating β cells are clustered in the core of a generally round islet, surrounded by a mantle of α, δ, and PP cells (Cabrera et al., 2006). While some studies suggest that the tail (or splenic part) of the pancreas contains the highest concentration of islets (Elayat et al., 1995), others had found that islet size and distribution are similar in different regions of the adult mouse pancreas (Kim et al., 2009). On the other hand, the human islets lack the typical core–mantle architecture of rodent islets and β cells are located in small, core-like clusters intermingled with the other kinds of endocrine cells (Brissova et al., 2005). Islet cells may interact with each other through direct contact and through systemic or paracrine effects. The highly specialized pattern of blood flow through the islet probably controls local and systemic interactions. Although the islets constitute only a minor part of the total pancreatic mass (1 %), they receive about 15-20 % of total pancreatic blood flow and this portion is further increased when glucose level is increased (Ballian and Brunicardi, 2007; Nyman et al., 2010). This effect is thought to be mediated by interactions between the parasympathetic nervous system and locally produced metabolites, mainly adenosine (Carlsson et al., 2002). However, Jansson et al. (2008) reported that increased insulin secretion in response to increased glucose concentration was without concomitant increase in islet blood flow. The effects of anti-diabetic agents on islet blood flow were investigated in a few studies using the microsphere technique in anesthetized rats. However, conflicting results were reported for sulfonylureas, e.g., tolbutamide increased islet blood flow (Vetterlein et al., 1985), whereas glipizide decreased it (Jansson et al., 2003). On the other hand, α receptor blockers appear to suppress the hyperglycemia-induced islet hyperperfusion, which may ameliorate hemodynamic stress in pancreatic islets (Iwase et al., 2009).

1.1.3 The β cell

The β cell is the most abundant cell type in the islet of Langerhans and solely builds up its core. Under experimental conditions β cells can be discriminated morphologically from other types by their ellipsoid shape and bigger size. Furthermore, different electrophysiological properties also enable to distinguish β cells from non β cells (Gopel et al., 1999). The different cell types of the pancreatic islets are regulated by complex para- and autocrine interactions that are yet not fully understood in their characteristics and functions. Insulin or other secretory products of β cells like γ aminobutyric acid (GABA) or zinc can inhibit α (Rorsman et al., 1989; Ishihara et al., 2003; Ravier and Rutter, 2005; Franklin et al., 2005) and δ cells (Rouiller et al., 1981), whereas glucagon release from α cells stimulates both insulin (Samols et al., 1965) and somatostatin (Patton et al., 1977) secretion. The latter, in turn, prominently inhibits both β and α cells (Strowski et al., 2003). Proinsulin, the precursor of insulin, is synthesized in the endoplasmic

reticulum and undergoes a series of maturation steps, starting already in the Golgi. The product is then packaged into secretory granules that gradually acidify, allowing further processing to insulin (Hutton, 1994). The resulting insulin has a lower solubility and so it co-precipitates with zinc ions to form microcrystals within the secretory granule (Orci et al., 1986).

1.2 Physiology of the Pancreas

1.2.1 Stimulus-secretion coupling

The term "stimulus secretion coupling" in the endocrine cells was developed by Douglas and Rubin (1963). It is now being widely accepted, that stimulus secretion coupling in β cells is achieved by at least two interconnected pathways for which the two terms "K_{ATP} channel dependent" or "triggering" pathway and 'K_{ATP} channel-independent' or "amplifying" pathway are in common use today (Henquin, 2000).

1.2.1.1 The triggering pathway

ATP-sensitive K^+ (K_{ATP}) channels are key players in the triggering pathway (Henquin, 2000). The β cell K_{ATP} channel is a hetero-octameric complex formed from four inwardly rectifying potassium channel subunits (termed Kir6.2) and four sulphonylurea receptor subunits (SUR1). Both subunits are required to form a functional channel. The Kir6.2 subunits build up the channel pore through which potassium ions flow. The name of Kir came from the fact that these channels conduct K^+ current into the cell more easily than to the outside of the cell (reviewed in Hibino et al., 2010). SUR1 subunits surround the Kir6.2 subunits, is expressed at high levels in pancreatic islets and pancreatic β cell lines including MIN6 and RINm5F (Inagaki et al., 1995a). The activity of K_{ATP} channels is modulated by changes in the ATP/ADP ratio. SUR1 mediates the opening action of Mg^{2+}-ADP (Gribble et al., 1998), whereas the closing action of ATP is on Kir6.2 itself. SUR1 subunit, as implied by its name, binds sulphonylurea, which close the channel, while it is activated by diazoxide (Bryan et al., 2004). At non stimulatory glucose concentrations, K_{ATP} channels have a high opening probability leading to a net outflow of K^+ ions through spontaneously active K_{ATP} channels. With the K^+ gradients existing over the β cell membrane this results in a negative membrane potential of around -70 mV. An increase in the level of blood plasma glucose evokes the diffusion of glucose into the cytoplasm of the β cells by means of a high affinity membrane bound Glucose Transporter (GLUT1 in humans; GLUT2 in rodents) (Scheepers et al., 2004; Thorens and Mueckler, 2010). Glucose is rapidly phosphorylated to glucose 6-phosphate by glucokinase, which thereafter acts as the glucose sensor of the β cells. Subsequent oxidative metabolism provides pyruvate which is channelled into the mitochondria via Krebs cycle, resulting in the production of NADH and $FADH_2$. The

resultant increase in the ATP/ADP ratio in the cytosol causes depolarization of the plasma membrane by closure of the K_{ATP} channels. This permits opening of voltage - dependent Ca^{2+} channels (VDCC) accompanied by release of Ca^{2+} into cytosol and subsequently, Ca^{2+} dependent action potential firing is initiated. Native pancreatic β cells and cell lines have different types of VDCC. These channels are classified according to their open probability in low-voltage-activated (LVA, type T channels) or high-voltage-activated (HVA, L, N, P/Q, and R). In both types, they all form heteromeric complexes composed of α 1, α 2, β, and γ subunits. L-type Ca^{2+} channels are considered the most important Ca^{2+} entry pathway that controls insulin secretion in primary β cells and cell lines (Yang and Berggren, 2006; Hiriart and Aguilar-Bryan, 2008). It has been shown that the increase of β cell calcium channel activity and / or density results in enhanced insulin exocytosis (Yang and Berggren, 2005). Moreover, these channels are also involved in β cell development, survival, and growth (Namkung et al., 2001). The voltage gated K^+ channels, are responsible for repolarization of the pancreatic β cells (MacDonald et al., 2001). The subsequent increase in cytosolic free Ca^{2+} coupled with the multiple phosphorylation events modulated by protein kinase C (PKC) and protein kinase A (PKA) induce exocytosis and insulin secretion (Ashcroft et al., 1994). This general model of glucose stimulated insulin secretion (GSIS) is widely accepted today especially because most experimental conditions that interfere with the rise in $[Ca^{2+}]_i$ impair glucose-induced insulin secretion, whereas all agents, either physiological or pharmacological, that increase β cell $[Ca^{2+}]_i$ induce insulin secretion (Henquin, 2000 and 2004). However, if $[Ca^{2+}]_i$ is accompanied by a reduced ATP level, it is unlikely to elicit a secretory response (Rustenbeck et al., 1997a).

1.2.1.2 The amplifying pathway

While evidence supporting the essential role of K_{ATP} channels in glucose-induced insulin secretion was accumulating, the possibility that glucose had additional independent effects was expressed by investigators many years ago. One study showed that 15 mM glucose alone induced a higher rate of insulin secretion than a combination of 10 mM glucose and 5 μM tolbutamide, although both conditions produced similar electrical activity and, presumably, a similar increase of $[Ca^{2+}]_i$ in β cells. It was therefore proposed that non-electrogenic effects of glucose amplify insulin secretion (Henquin, 1988). Another study reported that glucose was able to increase insulin secretion, in the presence of sulfonylurea concentrations sufficient to close all K_{ATP} channels leading to the proposal that glucose was acting on other targets (Panten et al., 1988). Two independent studies (Gembal et al., 1992; Sato et al., 1992) had shown that glucose-independently of K_{ATP} channels was still able to evoke an increase in insulin secretion above the already raised basal level in mouse or rat islets depolarized by KCl in the presence of diazoxide

i.e. when K_{ATP} channels were held open pharmacologically. This effect of glucose was shown to depend on metabolism, to require elevated $[Ca^{2+}]_i$ (by KCl-induced depolarization in these experiments), but was not found to involve a further rise in $[Ca^{2+}]_i$ (Gembal et al., 1992). Moreover, glucose was recently reported to increase insulin secretion from mouse islets lacking K_{ATP} channels which confirm amplification to the signaling mechanism that circumvents depolarization–mediated effect (Nenquin et al., 2004; Ravier et al., 2009). Several approaches using intact or permeabilised cells have shown the involvement of ATP at steps of the secretory process distal to the $[Ca^{2+}]_i$ rise (Detimary et al., 1994; Rustenbeck et al., 1997a). Recent studies call for a evaluation of the roles of citrate cycle intermediates exported to the cytosol via cataplerosis (MacDonald et al., 2005), cAMP (Dyachok et al., 2008), NAD(P)H (Ivarsson et al., 2005; Reinbothe et al., 2009), AMP-activated protein kinase (Rutter and Leclerc, 2009).

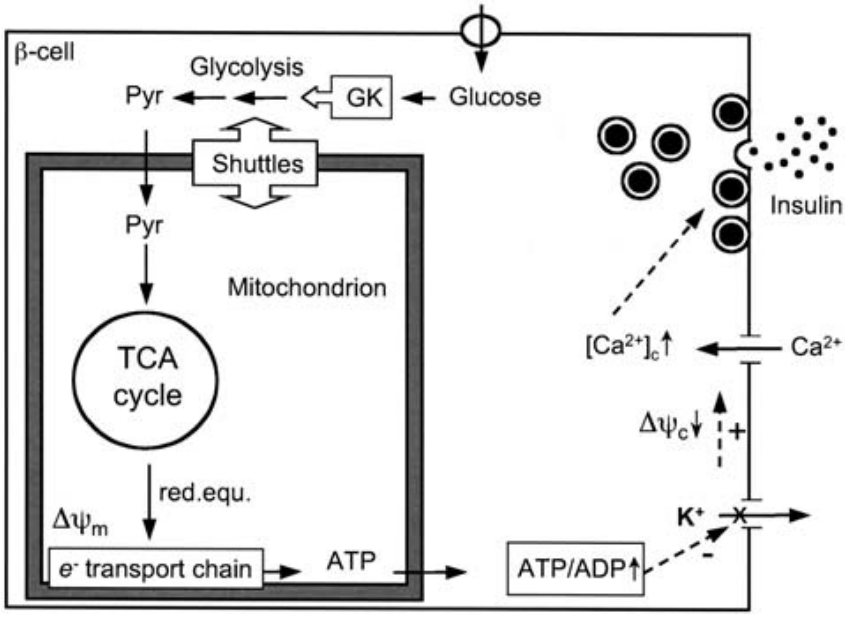


Figure 1.1 Model for coupling glucose metabolism to insulin secretion in the β cell. Glucose equilibrates across the plasma membrane and is phosphorylated by glucokinase (GK), which initiates its conversion to pyruvate (Pyr) by glycolysis. Pyr preferentially enters the mitochondria and fuels the TCA cycle, resulting in the transfer of reducing equivalents (red.equ.) to the respiratory chain, leading to hyperpolarization of the mitochondrial membrane (ΔΨm) and generation of ATP. ATP is then transferred to the cytosol, raising the ATP/ADP ratio. Subsequently, closure of K_{ATP} channels depolarizes the cell membrane. This opens voltage-gated Ca^{2+} channels, increasing the cytosolic Ca^{2+} concentration $(Ca^{2+})_i$, which triggers insulin exocytosis (Maechler, 2002).

1.2.2 Neuronal regulation of insulin release

Both parasympathetic and sympathetic nerves have an important effect on islet secretory activity. It is thought that feeding induces the parasympathetic nervous system (rest and digest mood), which facilitates insulin secretion in a glucose-dependent manner (Brunicardi et al., 1995). Pancreatic ganglia are supposed to govern the synchronicity of insulin secretion between islets observed over the whole pancreas (Stagner and Samols, 1985). Furthermore, sympathetic activity (fight and flight mood) was reported to regulate the homeostasis of glucose by adjusting

insulin secretion to stressful conditions including exercise (Järhult and Holst, 1979). The islets are densely innervated by nerve fibers, which are closely related to blood vessels terminating close to islet cells (Burris and Hebrok, 2007). Studies on the islet innervating fibers with histochemical and fluorescence staining revealed not only nerves containing the classical neurotransmitter acetylcholine and norepinephrine (Coupland, 1958; Cegrell, 1968), but also a variety of established and putative neuropeptides (Boonen et al., 2007). Pancreatic islets are abundantly innervated by parasympathetic nerves (from the vagus nerve) releasing acetylcholine which enhance insulin secretion in different species (Ahrén, 2000; Gilon and Henquin, 2001). Among the five different muscarinic receptors, only the M3 in mice was found to be important for the cholinergic stimulation of glucagon and insulin secretion (Duttaroy et al., 2004; Gautam et al., 2008). Catecholamines have a dual effect on the β cell to induce either inhibition or stimulation of insulin secretion through their interaction with α2 or α1 and β2 adrenergic receptors, respectively (Skoglund et al., 1986; Savontaus et al., 2008). Apart from parasympathetic and sympathetic nerves, several other fibers innervate the islets although their contributions to control islet hormone secretion are unclear. Neuropeptides were discovered in nerve terminals in pancreatic ganglia and in islets in several species: vasoactive intestinal polypeptide (VIP), gastrin releasing peptide (GRP) and pituitary adenylate cyclase activating polypeptide (PACAP). These neuropeptides are released from the pancreas on electrical stimulation of the vagus and stimulate both insulin and glucagon secretion (Fridolf et al., 1992; Winzell and Ahrén, 2007). Other neuropeptides including calcitonin-gene related peptide (CGRP) and substance P are thought to be involved in the synthesis and/or release of insulin from secretory granules (Edwin and Leigh, 1999; Adeghate and Ponery, 2003).

1.3 Fluoroquinolone antibiotics

1.3.1 History and Classification

Quinolones are widely used antimicrobial agents. The development of these drugs started with the non-fluorinated drug nalidixic acid in the early 1960s and proceeded in the 1980s to the first 6-fluorinated derivatives with enhanced activity against Gram-negative bacteria (e.g. norfloxacin, ofloxacin, ciprofloxacin). At that time, it was generally believed that fluorination is essential to obtain drugs with high anti-bacterial activity. Later on, fluoroquinolones with improved activity against Gram-positive bacteria (e.g. moxifloxacin, gatifloxacin) have come to therapeutic use. Recently, highly active quinolones have been developed which are not fluorinated in position 6, but bear fluorine atoms at the side chain (e.g. BMS-284756) or are non-fluorinated derivatives (e.g. PGE 9509924). Thus, some prefer to avoid the term fluoroquinolones and rather call them more generally ‘quinolones’, although they differ

considerably from the classic drugs such as nalidixic acid. With the given multiplicity of quinolones, it is necessary to group them according to their features in different classes as has been proposed by the Paul–Ehrlich-Society of Chemotherapy (PEC). Those fluoroquinolones that are most often used today for the treatment of bacterial infections, mainly urinary tract infections and respiratory tract infections, and that are considered relatively safe and well-tolerated drugs are compiled in Table 1.1 (Naber and Adam, 1998; Stahlmann, 2002). Compared to other antibiotics, fluoroquinolones have rapid bactericidal effect with good oral absorption, prolonged half-lives, and efficacy, concentrated in tissues at levels that often exceed their serum concentrations. They exert their bactericidal effect by interfering with DNA topoisomerase II and IV (Mitscher, 2005).

PGE 9509924

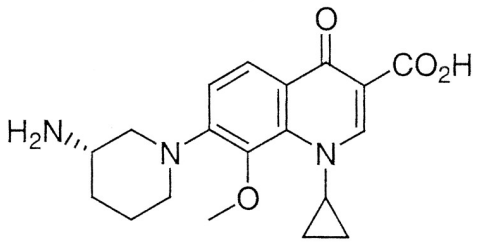


Table 1.1 Widely used fluoroquinolones grouped according to the PEC classification

Class	Fluoroquinolone	Spectrum / therapeutic use
I	Norfloxacin	Mainly active against Gram negative pathogens, almost exclusively used for urinary tract infections
II	Ofloxacin and Ciprofloxacin	Broad indications for systemic use, but moderate activity against Gram positive pathogens
III	Levofloxacin	Improved activity against Gram positive and atypical pathogens, infections of the respiratory tract and others
IV	Moxifloxacin and Gatifloxacin	Improved activity against Gram positive and atypical pathogens as well as anaerobes, infections of the respiratory tract and others

1.3.2 Special uses

Generally, fluoroquinolone use for children has been avoided, except in circumstances where alternative antibiotic choices are limited (Sabharwal and Marchant, 2006) and the benefits clearly outweigh the risks of therapy e.g. Anthrax prophylaxis or infection, complicated urinary tract infection caused by multidrug-resistant, shigellosis (WHO, 2005; Traa et al., 2010), Gram negative bacteria (FDA approved; Committee on Infectious Diseases, 2006). Otherwise, they are avoided because of concerns of emergence of bacterial resistance and arthralgia (Chalumeau et al., 2003; Murray and Baltimore, 2007). Other, not yet approved indication; Typhoid and paratyphoid fevers (Leibovitz, 2006), Gram negative neonatal sepsis/meningitis and chronic or acute osteomyelitis or osteochondritis (Velissariou, 2006). Among the various fluoroquinolones, ciprofloxacin is the most potent against pseudomonas and most safe and effective drug in children and neonates (Ahmed et al., 2006).

The use of fluoroquinolones during the first trimester of pregnancy had not been associated with increased risk for malformations, stillbirths, preterm births or low birth weight (Bar-Oz et al., 2009). Although both levofloxacin and moxifloxacin have been classified as category C (There is a positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk), they have high transplacental passage rates (Ozyuncu et al., 2010).

1.3.3 Fluoroquinolones withdrawn from the market

Owing to multiple adverse effects including severe anaphylaxis, tendon rupture, and cardiotoxicity several fluoroquinolones have been withdrawn (e.g. temafloxacin and grepafloxacin) or strictly limited in their uses (e.g. trovafloxacin) after marketing (Table 1.2 summarize fluoroquinolones withdrawn from the market). A serious idiosyncratic reaction profile is possibly related to the immunologically reactive 1-difluorophenyl substituent that characterizes temafloxacin, tosufloxacin, and trovafloxacin (Rubinstein, 2001). The withdrawal of temafloxacin in 1992, only 6 months after its introduction, followed the observation of serious adverse events that were labeled the ‘‘temafloxacin syndrome’’ (Blum et al., 1994). It consists of hemolysis, renal dysfunction, hemolytic anemia, anaphylaxis and hepatic dysfunction. In 1999, trovafloxacin was withdrawn after reports of hepatotoxicity in which some cases required liver transplantation (Ball et al., 1999). Before this development, several studies had been published describing impressive clinical efficacy coupled with a good safety profile (Williams and Hopkins, 1998). Grepafloxacin was withdrawn from the market in 1999 because of its arrhythmogenic effect (Haverkamp et al., 2000). Clinafloxacin has been withdrawn because of phototoxicity and hypoglycemia and sparfloxacin because of phototoxicity (Zhanel et al., 2002).

Table 1.2 Twelve fluoroquinolones which are no longer used or have limited significance due to their specific toxicities.

Fluoroquinolone	Year of withdrawal	Rationale for withdrawal
Enoxacin	1985	Inhibition of cytochrome p 450
Pefloxacin	1985	Tendopathies and phototoxicity
Fleroxacin	1990	Phototoxicity and CNS effects
Sitafloxacin	1991	Phototoxicity
Temafloxacin	1992	Hemolytic uremic syndrome, hypoglycemia in elderly patients, renal failure, and coagulopathy
Lomefloxacin	1993	Phototoxicity
Sparfloxacin	1994	Phototoxicity and QT prolongation
Trovafloxacin	1999	Hepatotoxicity and CNS effects
Grepafloxacin	1999	QT prolongation and arrhythmia
Clinafloxacin	1999	Phototoxicity, hypoglycemia, and inhibition of cytochrome p 450
Gatifloxacin	2006	FDA Announces Label and indication changes as a result of continued reports of serious cases of hypoglycemia and hyperglycemia

1.3.4 Specific toxicities of fluoroquinolones

In the following section, some toxic effects of fluoroquinolones will be discussed as reported in the major reviews in this field. Information on undesired effects can be obtained from different sources including clinical trials, postmarketing surveillance, and animal studies. Adverse Events attributable to moxifloxacin was clinically higher than was the rate for any other fluoroquinolone (Shehab et al., 2008).

1.3.4.1 GIT

These include diarrhea, nausea, vomiting, distorted taste sensation and occasional abdominal pain. Fluoroquinolones, especially C8-methoxy fluoroquinolones, such as moxifloxacin and gatifloxacin, have been incriminated to be the most commonly implicated antibiotics in Clostridium difficile-associated colitis (CDAD) cases (Hookman and Barkin, 2009). Calculated attack rates were higher for the gatifloxacin than the levofloxacin-associated cases (30% versus 17% respectively) (Gaynes et al., 2004). Increased incidence of CDAD had caused the development of fluoroquinolone-resistant strains (Muto et al., 2005; Loo et al., 2005). On the contrary, recent study showed no significant correlation between fluoroquinolone utilization and development of CDAD (Novell and Morreale, 2010). Hepatotoxicity associated with the fluoroquinolones has become a concern with gatifloxacin (Henann and Zambie, 2001) and ciprofloxacin (Zimpfer et al., 2004); even levofloxacin had been associated with hepatic failure (Spahr et al., 2001; Coban et al., 2005). On the contrary, other fluoroquinolones were frequently used to replace agents in first line anti-tuberculosis regimens in patients with TB who have drug-

induced hepatic dysfunction (Ho et al., 2009). The safety of moxifloxacin was reviewed in the EU and Canada following concerns over liver safety (European Medicines Agency 2008; Health Canada, 2010).

1.3.4.2 Cardiac

The impact of fluoroquinolones usage on the onset of arrhythmia has been investigated by a few epidemiological studies (Clark et al., 2001; Corrao et al., 2006; Raschi et al., 2009). Prolongation of the corrected QT interval (QTc) is a well-known adverse effect associated with the use of fluoroquinolones, which can lead to potentially life-threatening arrhythmias such as Torsades de pointes (Tdp). This is characterized by beat-to-beat variation in the QRS complexes in any of the electrocardiogram (ECG) leads and occurs at rates of 200-250/ min. The clinical presentation includes light-headed spells, near syncope, syncope and may progress to cardiac arrest (Falagas et al., 2007). Risk factors for Tdp include female gender, history of familiar long QT syndrome, organic heart disease and congestive heart failure, bradycardia, electrolyte imbalance (especially hypokalemia, hypomagnesemia and hypocalcemia), renal failure, and hepatic dysfunction (Zeltser et al., 2003). The cardiotoxic effect is mainly mediated by blocking the cardiac voltage-gated potassium channels, particularly the rapid component (I_{Kr}) of the delayed rectifier potassium current. Inhibition of I_{Kr} delays cardiac repolarization by blocking outward flow of potassium ions from myocytes. I_{Kr} is encoded by human ether-a-go-go-related gene (HERG). The blocking ability is not the same for all fluoroquinolones being structurally related. The most potent inhibitor is sparfloxacin (Zünkler et al., 2006), moxifloxacin and gatifloxacin have an intermediate inhibitory effect on the (HERG) channel current, while levofloxacin and ciprofloxacin have the least inhibitory effects (Kang et al., 2001). Falagas et al. (2007) had reported that levofloxacin and gatifloxacin were associated with significantly higher rates of Tdp, however still moxifloxacin appears to have the greatest potential to cause QT prolongation (Badshah et al., 2009; Malik et al., 2009). Gemifloxacin, ofloxacin and ciprofloxacin possess a less pro-arrhythmogenic effect (Anderson et al., 2001; Bischoff et al., 2000; Kang et al., 2001; Yap and Camm, 2003; Sherazi et al., 2008). ECG data had shown that prulifloxacin is considered to devoid from any potential cardiotoxic effects (Rosignoli et al., 2010).

1.3.4.3 Neuropsychiatric complications

The fluoroquinolones are an under-recognized cause of drug-induced mental status changes (Farrington et al., 1995). Psychiatric disorders had been reported in few studies (Leone et al., 2003; Doussau de Bazignan et al., 2006). The overall incidence of CNS disorders associated

with fluoroquinolones is 1–3% (Thomas, 1994). Of these, the most commonly reported symptoms have included dizziness, headache, and somnolence. Other, less commonly reported symptoms include agitation, delirium, confusion, acute organic psychosis, abnormal vision (Lipsky and Baker, 1999) and suicidal ideation (LaSalvia et al., 2010). Risk factors involve age, compromised renal function, history of seizures, and co-administration with theophylline (Vancutsem and Schwark, 1992) or nonsteroidal anti-inflammatory drugs (Rollof and Vinge, 1993; Quigley and Lederman, 2004). Ciprofloxacin has been linked to a variety of neurologic side effects such as abnormal dreams, tremor, vertigo, alcohol intolerance, ataxia, migraine, status epilepticus, peripheral neuropathy, and oculogyric crisis (Azar et al., 2005), psychosis had been reported even after topical use (Tripathi et al., 2002). However, it is rarely involved in seizures (Jick et al., 1993; Agbaht et al., 2009), or hallucinations (Mulhall and Bergmann, 1995). Cases involving gatifloxacin- induced psychosis or delirium in elderly patients, possibly suggests that this age-group is more vulnerable (Reeves, 2007). Abnormal thinking and hallucinations are very rare (Blondeau, 1999; Perry et al., 1999). Fleroxacin had been associated with unacceptable frequencies (exceeding rates of 70%) of CNS-related adverse events (Bowie et al., 1989).

The incidence of seizures associated with fluoroquinolones varies among the individual agents and is relatively rare. Sitafloxacin and levofloxacin have a weak convulsant activity that is not enhanced by concurrent administration with anti-inflammatory drugs (Hori, 2009). The exact mechanism by which quinolones induces seizures is controversial. The pathophysiological basis for the triggering of seizures probably lies in the binding of fluoroquinolones to GABA receptors in the brain resulting in nervous system stimulation (Akahane et al., 1989; Domagala, 1994; Kushner et al., 2001). Binding to this receptor is strongly influenced by the side chain in the 7-position (7-piperazine e.g., ciprofloxacin, enoxacin, and norfloxacin) and those containing 7-pyrrolidine (e.g., tosufloxacin and clinafloxacin). Quinolones with bulky moieties, such as temafloxacin and sparfloxacin, bind less efficiently to GABA receptors (Owens and Ambrose, 2000; Akahane et al., 1989). Also, fluoroquinolones are thought to activate N-methyl-D-aspartate (NMDA) channels by chelating with magnesium and removing its channel blocking effect (Akahane et al 1993; Schmuck et al., 1998). It is also suggested that some fluoroquinolones (gatifloxacin, ciprofloxacin and levofloxacin) significantly reduced brain glucose uptake, as suggested by reducing GLUT1 mRNA expression, cell surface GLUT1 protein expression and glucose transport in HepG2 cells (Ge et al., 2007 and 2009). The possibility of seizures initiated by levofloxacin is increased when patients are taking other medications with epileptogenic properties that are CYP1A2 substrates, such as mirtazapine, metoclopramide, and theophylline (Bellon et al., 2009).

1.3.4.4 Ocular toxicity

Ocular toxicity appears to be dose-dependent and results from specific fluoroquinolone structures. Adverse effects, such as local irritation, stinging, chemosis, conjunctival hyperemia and superficial punctate keratitis tend to be relatively innocuous. However, reversible corneal precipitation, typically seen with hourly topical administration, may not be so benign. While advantageous in providing a depot of slow releasing drug, delayed corneal healing and perforation are serious complications that may occur in approximately 10% of cases (Thompson, 2007). Less severe, oedema of the corneal stroma occurred following topical administration and intravitreal injection of greater than 100 μg ciprofloxacin (Stevens et al., 1991). Although reported in animals and their potential contribution to early cataract (Zhao et al., 2010), no reports of fluoroquinolone-induced cataracts in humans (Thompson, 2007). The intracameral use of moxifloxacin was not associated with ocular toxicity (Kernt et al., 2009).

1.3.4.5 Myasthenia gravis

There have been case reports in which various fluoroquinolones except prulifloxacin (Rossi et al., 2009) have caused exacerbations of myasthenia gravis in patients with this underlying disease (Rauser et al., 1990; Mumford and Ginsberg, 1990; Vial et al., 1995). In these cases, muscle weakness appeared or worsened shortly after the initiation of fluoroquinolone treatment and improvement occurred after drug discontinuation and, in some cases, additional treatment was needed (Moore et al., 1988; Roquer et al., 1996).

1.3.4.6 Phototoxicity

Phototoxicity is a dermatologic complication of quinolone therapy ranging from mild to severe reactions that usually occurs within hours of exposure to an UV light source. Singlet oxygen molecules and other free radicals are generated when UV light comes in contact with certain quinolone compounds. Under these circumstances, the resulting products were believed to attack cellular components, including lipid membranes, resulting in a severe inflammatory process (Klecak al., 1997; Tokura, 1998). These reactions were more commonly associated with the so-called double-halogenated quinolones, namely lomefloxacin, sparfloxacin and sitafloxacin (Domagala, 1994; Owens and Ambrose, 2000; Stahlmann, 2002). Other agents were abandoned during development (e.g. BAY y 3118 and clinafloxacin) because of photoxicity. Phototoxicity is an uncommon adverse reaction with gatifloxacin or moxifloxacin, perhaps due to the presence of the 8-methoxy group (Marutani et al., 1993). The photosensitizing effect of grepafloxacin is relatively weak and similar to that of ciprofloxacin (Stahlmann and Schwabe, 1997).

1.3.4.7 Tendinopathy

The prevalence of the fluoroquinolones – induced tendon injury reported is low (0.14% - 0.4%) in an otherwise healthy population (Khaliq and Zhanel, 2005). The fluoroquinolones most commonly implicated are pefloxacin (37%) and ciprofloxacin (26%) (Salvi et al., 2007). Animal studies showed that the propensity to induce tendon lesions varies among fluoroquinolones. Fleroxacin, pefloxacin, levofloxacin, and ofloxacin were the most toxic, while enoxacin, norfloxacin, and ciprofloxacin had little or no effect (Kashida and Kato, 1997). In the Netherlands, a large simultaneous increase in non-traumatic tendon ruptures and fluoroquinolone use was observed in the period from 1991 to 1996 (van der Linden et al., 2001). Predisposing factors include advanced age > 60 years old, chronic lung diseases, diabetes mellitus, renal failure, gout, hypercholesterolemia, hyperparathyroidism (male predominance), low bone mineral density associated with osteoporosis, Magnesium deficiency (alcoholism, dietary and renal disease), rheumatoid arthritis, and corticosteroid therapy (Childs, 2007). However, cases were reported with levofloxacin with no predisposing factors (Gottschalk and Bachman, 2009; Durey et al., 2010). Tendon injury is usually presented by sudden onset of severe pain, tenderness, oedema, difficulty with movement of the involved area. It has been reported to occur as early as 2 hours after the first dose to as late as 6 months after treatment has been terminated (Casparian et al., 2000). The Achilles tendon is the most common site of injury, cited in about 90 % of patients, about 40 % bilateral. Other cases also include, patella, quadriceps, the hand (flexor tendon sheath and the extensor tendons of the fingers and the thumb) (McGarvey et al., 1996). The pathogenesis of quinolone-induced tendinopathy was considered to be multifactorial. Theories postulating the causes of tendinopathy were ischemic influences, matrix degrading phenomenon, and toxic changes on collagen fibers (Kowatari et al., 2004; Gold and Igra, 2003).

1.3.4.8 Arthropathy

Arthropathy has been reported with various fluoroquinolones, particularly in children where ciprofloxacin and pefloxacin were involved (Grady, 2005). However, ciprofloxacin did not cause clinical arthropathy and growth impairment even at 1 year of follow-up (Drossou-Agakidou et al., 2004). The lower chondrotoxic risk associated with the use of quinolones in children explained by some authors as differences in velocity of growth in experimental animals and human (Sendzik et al., 2009). So, fluoroquinolones are currently reserved for use in children as mentioned before (1.3.2).

1.3.4.9 Anaphylaxis

Interestingly, in a considerable number of cases, anaphylaxis was reported to occur after the first intake of a particular fluoroquinolone. In particular, ciprofloxacin (received marketing authorisation in the USA in 1987) by 1988, a total of 15 cases of anaphylaxis or anaphylactoid reactions associated with its use had been received by the US FDA (Davis et al., 1989). Most patients are sensitized to more than one fluoroquinolone, suggesting an extensive cross-reactivity between the different molecules as all of them share a common core structure (Scherer and Bircher, 2005). Anaphylaxis (type I, Ig E-mediated reactions occurring within 1 hour of administration) was more rarely found with quinolones than with other antibiotics such as β lactams. Forms of type I reactions include urticaria, angioedema, and anaphylactic shock. The only definitive test for diagnosis is the challenge with the drug itself (Aberer et al., 2003), which is a risky proposition, because fatalities have been reported (Peters and Pinching, 1989) although basophil activation test showed to have a good sensitivity and specificity with quinolones (Aranda et al., 2010).

1.3.4.10 Immune-related idiosyncratic reactions

These include hemolytic – uremic syndrome, hemolytic anemia, thrombocytopenia, leucopenia, acute interstitial nephritis, acute hepatitis, acute cholestatic jaundice, Steven-Johnson (life-threatening condition affecting the skin in which cell death causes the epidermis to separate from the dermis) and Lyell syndromes (Toxic Epidermal Necrolysis), fixed drug eruption, cutaneous vasculitis, macula-papular exanthema, acute pancreatitis, serum-sickness like syndrome, angioimmunoblastic lymphadenopathy, acute exanthematous pustulosis, and eosinophilic meningitis (Campi and Pichler, 2003). Fixed drug eruptions have been observed with ciprofloxacin, tosufloxacin, norfloxacin, and ofloxacin (Hager et al, 2009). Single case reports exist on patients with toxic epidermal necrolysis or Stevens-Johnson syndrome attributed to different fluoroquinolones. Most often ciprofloxacin was the responsible agent, but also trovafloxacin, ofloxacin, and levofloxacin have been reported (Jongen-Lavrencic et al., 2003). Animal studies suggest that quinolones were assumed to stimulate both mast cells and basophils to release histamine (Scherer and Bircher, 2005). Gatifloxacin, although devoid of photosensitivity reactions, may initiate idiosyncratic hypersensitivity reaction to cause Radiation Recall Dermatitis (RRD) (Jain et al., 2008). Several cases of cutaneous vasculitis due to ciprofloxacin, ofloxacin, and levofloxacin drug therapy have been reported (Maunz et al., 2009).

1.3.4.11 Legal implications

Adverse drug reactions continue to cause injury and harm to patients regardless of careful evaluation, prescription, and drug administration. This problem did grow into a cottage industry for malpractice lawyers. What makes fluoroquinolones special is that side effects appear or worsen for even months after the drug has been discontinued. A good example is the musculoskeletal injury associated with fluoroquinolones. It is the responsibility of the physician to be aware of the risks of fluoroquinolones. A physician's best defense in case of malpractice or negligence is to demonstrate that practice was within a reasonable standard of care and that any deviation from approved indications was documented at the time of care with literature support. Action against the practitioner may also be minimized by ensuring adequate patient informed consent especially for patients with risk factors (Kaufman et al., 1994).

1.4 Fluoroquinolones and dysglycemia

The first case report of hypoglycemia was in a diabetic patient; associated with enoxacin (Kobayashi et al., 1991) followed by ciprofloxacin (Whiteley et al., 1993), lomefloxacin (Suda et al., 2000); and even hypoglycemic coma with norfloxacin (Haruhara et al., 2000). Oliphant and Green (2002) published the first review that acquaints gatifloxacin as a causative agent of dysglycemia if used concomitantly with antidiabetic agents. Further cases reported by Menzies et al. (2002), and Baker and Hangii (2002). This caused a revision of the package insert to include warnings of serious dysglycemias (Lewis-Hall, 2006). Levofloxacin was suspected for 22 domestic reports of dysglycemia from 1997 to 2006 in Canada (Hunt, 2007). As a consequence of hypoglycemia, some cases were complicated by anoxic brain injury (Lawrence et al., 2006) or by pontine myelinolysis and quadriplegia (Vallurupalli et al., 2008). Fatal hypoglycemia was reported with levofloxacin (Friedrich and Dougherty, 2004; Singh et al., 2008). Both hypoglycemia and hyperglycemia have been recognized with moxifloxacin use (Avelox®, package insert, 2002; Smith and Lomaestro, 2003). However, no life threatening reports of hypoglycemia were reported so far neither with gemifloxacin (Ball et al., 2004), nor with moxifloxacin (Gavin III et al., 2004). Donaldson et al. (2004) first correlated gatifloxacin and symptomatic severe hyperglycemia. This was followed by many case reports (Ambrose et al., 2003; Arce et al., 2004; Happe et al., 2004; Blommel and Lutes, 2005; Beste and Mersfelder, 2005; Yip and Lee, 2006; Ovartlarnporn and Jongjaroenprasert, 2007). Risk factors for hypoglycemia in patients without diabetes are older age, low plasma albumin concentration, liver disease, malignancy, chronic heart failure, and concomitant use of oral hypoglycemic (Owens, 2005). Risk factors for hyperglycemia may include diabetes, renal impairment (Mohr et al., 2008), sepsis (Orlander and Serrao, 2004), and old age (Biggs, 2003). While hypoglycemia

is very infrequent, as highlighted by the studies of Mohr et al. (2005) and Graumlich et al. (2005) , hyperglycemia occurring more often. As opposed to the timing of hypoglycemic events, review of several case reports revealed that fluoroquinolone-associated hyperglycemia has generally occurred later in treatment, usually after 4 days of therapy, and with higher doses (Catero, 2007). Fluoroquinolones, as well as other antibacterials have been reported with dysglycemia (hypo or hyperglycemia) in case reports and databases of adverse drug reactions (Basaria and Cooper, 2005; Strevel et al., 2006; Jose et al., 2007). Nevertheless, apparently the risk of a clinically relevant dysglycemia is variable among fluoroquinolones. The rates of severe hypo- and hyperglycemia were significantly greater with gatifloxacin 1.1% while for levofloxacin, ciprofloxacin (0.3% for each), and moxifloxacin (0.2%) (Park-Wyllie et al., 2006; Lodise et al., 2007; Aspinall et al., 2009; Van Bambeke and Tulkens, 2009). In phase II and III studies, clinafloxacin was associated with a 4% frequency of hypoglycemia versus 1.1% in comparator-treated patients (Garber et al., 2009). Spontaneous reporting of adverse drug reactions (ADRs) suffers from underreporting perhaps as newer agents are more likely than older agents to be reported due to enhanced prescriber and media attention which is known as the Weber effect (Hartnell and Wilson, 2004). Data source alone is unreliable to quantify risk assessment because preclinical testing, phase I-III trials, and postmarketing surveillance all have limitations in their ability to identify infrequently occurring toxicities (Owens and Ambrose, 2005). The Naranjo probability rating scale (Naranjo et al., 1981) revealed that fluoroquinolones was the probable cause in many cases (Zvonar, 2006). Therefore, glucose as well as drug monitoring is highly recommended in patients with long-term use of steroids, cyclosporine, and decreased creatinine clearance and diabetics (Khaira et al., 2009). The critical question is that fluoroquinolones per se are culpable of producing dysglycemia? The theory of a possible drug-drug interaction has been considered among many case reports involving fluoroquinolones and antihyperglycemic medications. Glyburide was the most often implicated antihyperglycemic drug, accounting for five of these cases. The remaining interactions involved glimepiride, pioglitazone (with glyburide), and repaglinide. However, only ciprofloxacin is completely metabolized by cytochrome P450 (CYP) which might explain severe hypoglycemia due to interaction with glyburide (Roberge et al., 2000; Lin et al., 2004; Kelesidis and Canseco, 2010). However, it was found that moxifloxacin, and gatifloxacin were negligible inhibitors to cytochrome P450 isoforms; CYP1A2 and CYP2C9 (Zhang et al., 2008). Still, severe and resistant hypoglycemia was attributed to interaction between gatifloxacin and sulfonylureas (LeBlanc et al., 2004). Dosage reduction seems as ineffective strategy to prevent the hypoglycemic event associated with gatifloxacin or levofloxacin in patients with renal

insufficiency (Garber et al., 2009). So, it is unlikely that the pharmacokinetics of these drugs could sufficiently explain the dysglycemic episodes.

Fluoroquinolones appear as indispensible antimicrobial agents despite of the emergence of resistance. Several agents had been examined as inhibitors of mycobacterial infections especially tuberculosis as one of the re-emerging diseases (Onyenwenyi et al., 2008; Senthilkumar et al., 2009). Though Bristol-Meyers Squibb has voluntarily withdrawn gatifloxacin from the US and Canadian drug markets (Lewis-Hall, 2006), it is still marketed in other parts of the world. So, it is very important to search for the possible mechanism clarifying the potentially life threatening dysglycemic episodes and also there is deep need for development of new compounds devoid of such effects.

2. Aim of work

Pancreatic β cells regulate the whole body metabolism by secreting the hormone insulin in response to raised levels of blood glucose. However, the mechanisms underlying (GSIS) are not completely understood. It is thought that some fluoroquinolones produce endocrine toxicity in the form hypoglycemia via blockade of K_{ATP} channels. Gatifloxacin, in particular has been shown to produce both hypo- and hyper-glycemia. So, the aim of the present study is to answer the following questions:

i. Is that toxicity related to change in cellular calcium level, as can be expected from K_{ATP} channel blocking agents? If so, could that lead to β cell death (cytotoxicity)?
ii. Is there any peculiar morphological feature for β cells and /or pancreatic islets that could be attributed to fluoroquinolones toxicity?
iii. Is that combination of hypo- and hyper-glycemia specific toxicological feature for gatifloxacin or could be induced by other fluoroquinolones?

3. Materials and methods

3.1 Equipment

Equipment	Model	Company
Water purification system	Ultra clear Arium 611 VF	Sartorius
Autoclave	Vx-120 and DX-90	Systec GmbH
Balance	BP 210 D	Sartorius, Germany
pH meter	766 Climatic	Knick
Centrifuge	Biofuge Primo R Benchtop	Heraeus
Sterilizer (Oven)	MOV 212	SANYO
Balance	BP 210 D	Sartorius, Germany
Heated Circulator water bath	1003	GFL,GmbH
Clean bench	Laminar Air HBH	Heraeus
Magnetic rotator	RCT basic	IKA-Labortechnik
Mini-shaker	MS1	IKA
CO2 incubator	MCO-18AIC	SANYO
Stereomicroscope	STEMI 2000 with KL 1500 light source	Carl Zeiss
Shaking Water bath	ST1-338	Edmund Buehler
Peristaltic pump	Varioperpex II 2120	LKB Bromma
Dichroic beam splitter	TK 405	Leitz
Photon counting unit (Power supply, pulse forms unit, and CTM-05 counting board)	3460-04	Hamamatsu Photonics
Compound microscope	Orthoplan	Leica/Leitz
Cooled digital CCD camera		Theta electronics GmbH
Mercury arc lamp	ST41	Heraeus –Hanau
Xenon lamp	L2274	Hamamatsu

3.2 Basic medium

3.2.1 Krebs-Ringer-Bicarbonate-Medium

The basic medium for the isolation and the preparation of the pancreatic islets as well as the following dissociation into single β cells is a modified Krebs-Ringer-Bicarbonate-Buffer abbreviated as mKRB (Panten et al., 1977). The modification consists of the addition of HEPES solution which provides rather steady pH of 7.4 for a longer period. Lernmark had shown that the substitution of an adequate portion of the necessary bicarbonate concentration by the organic HEPES buffer prevented the alkalinisation of the medium as a result of the release of CO_2 (Lernmark, 1974)

For the preparation of 2 L of mKRB, the following amounts of organic matters and inorganic salts were dissolved in autoclaved pyrogen- free deionised water to reach the final concentration shown in the right column.

1) 752.74 mg $CaCl_2 * 2\ H_2O$ (Merck, MW 147.02)	2.5 mM
2) 700.77 mg KCl (Merck, MW 74.55)	4.7 mM
3) 326.26 mg KH_2PO_4 (Merck, MW 136.09)	1.2 mM
4) 591.55 mg $MgSO_4 * 7\ H_2O$ (Merck, MW 246.48)	1.2 mM
5) 13.44 g NaCl (Roth, MW 58.44)	115 mM
6) 3.36 g $NaHCO_3$ (Merck, MW 84.01)	20 mM
7) 1.98 g D-Glucose monohydrate (Merck, MW 198.17)	5 mM
8) 4.76 g HEPES (Roth, MW 238.31)	10 mM
9) 2.0 g Albumin (Serva, MW 66382)	0.1 % (w/v)

Albumin was added lastly to avoid excessive foam formation during stirring. The pH value of the resultant mKRB was adjusted to 7.4 by using 1N NaOH solution and was filtered under sterile conditions (Prefilter: 13400-130-K; Main filter: 11107-142-G, Sartorius).

The resultant mKRB was filled into sterile containers of 100 ml portion and stored at a temperature of 2 - 8 °C for a period maximally 4 weeks. Since prolonged storage can alter pH value, it is worthwhile to readjust pH before use using 1N NaOH or 1N HCl solutions.

3.2.2 Solutions for islets and islet cells isolation and culture medium

3.2.2.1 Sodium hydroxide-solution 1 N

Sodium hydroxide 40 g (Merck, MW 40) completely dissolved in a volume of 1L distilled water.

3.2.2.2 Collagenase P-solution

Collagenase P is prepared from the extracellular culture filtrate of a special Clostridium histolyticum strain. It is supplied as lypholisate and has a considerable lot to lot variability of the enzymatic activity (Yamamoto et al., 2007). To optimize islet isolation, both the amount of collagenase as well as period of digestion were adjusted for every batch (de Haan et al., 2004). Depending on the activity of the used batch, 1.0 -1.5 mg / 2 ml solution was used of collagenase P (Serva Electrophoresis GmbH, group I). To keep it stable, the enzyme was stored at a temperature of 2 °C and immediately before usage, dissolved in the suitable volume of freshly prepared mKRB (Bucher et al., 2004).

3.2.2.3 Ca^{2+} -free solution for the dissociation of the isolated islets into β cells

The contents of the Ca^{2+} free medium are the same as for mKRB except for the omission of $CaCl_2$ and the addition of 19.02 mg EGTA (Sigma, MW 380.4) to reach a ready to use concentration of 0.5 mM.

3.2.2.4 Collagen-solution used for the islets culture

The cover slips (diameter 25 mm, thickness 0.13 - 0.17 mm, Menzel, Braunschweig) were first rinsed with distilled water and left to dry, and then sterilized by heating for 40 minutes at 180 °C. For cultivation of pancreatic islets, collagen solution (1.2-1.3 µl) was evenly distributed on middle of cover slips. For preparation of 2 ml of collagen solution, 1 mg of collagen type 1 (Sigma) was weighed in a sterilized Eppendorf cup to be dissolved in a sterile 0.2 % acetic acid immediately sterilized before addition using a Filtropur 0.2 S syringe filter (Sarstedt, Germany) (Lenzen et al., 2000). Subsequently, the collagen was dissolved in an ultrasonic bath. The final solution was stored at 4 °C.

3.2.2.5 Culture medium RPMI 1640

Studies of optimal culture conditions demonstrated that RPMI 1640 with serum maintains glucose-stimulated insulin secretion in murine islets (Andersson, 1978). RPMI contains inorganic nutrient salt and buffer systems, vitamins, provitamins, as well as essential amino acids, Phenol-red and Glutathione. During the first two hours in cell culture, (the initial adhesive phase) the pancreatic islets were incubated with RPMI medium containing 10 mM glucose. For

1 L of solution to be used, 8.44 g RPMI 1640 (Gibco), 1.98 g glucose and 2.0 g $NaHCO_3$ (Merck) were dissolved in 0.9 L distilled water. The pH was adjusted further if necessary by adding either 1N HCl or 1N NaOH until pH 7.2 was obtained. Penicillin and streptomycin were added to give a final concentration of 100 IU / ml penicillin and 100 µg/ml streptomycin (Gibco). After setting the pH at 7.2, the medium was immediately filtered using a Sartorius Vacuum flask filter system with a Sartorius glass prefilter (Prefilter: In 13400-130- K, 0.2 µm Sartorius Cellulose acetate filter: 11107-142-G, Sartorius) and stored at 2 – 8 °C. The resultant RPMI 1640 medium was dispensed into 90 ml sterile bottles. Immediately before use, the medium was supplemented by 10 ml of a heat-inactivated fetal calf's serum (FCS) (Gibco) with change of pH to 7.4. In case of the pancreatic islets, 2.5 ml of RPMI medium (5 mM glucose) was added after a period of 3-4 h.

3.2.2.6 Culture medium RPMI 1640, modified with 5 mM glucose

D-glucose monohydrate (99.1 mg) was dissolved with 844.0 mg of RPMI 1640 glucose-free as well as 2.0 mg $NaHCO_3$ (Merck) in a volume of 90 ml distilled water. Further conditions for preparation and storage were the same as those mentioned in 3.2.2.5.

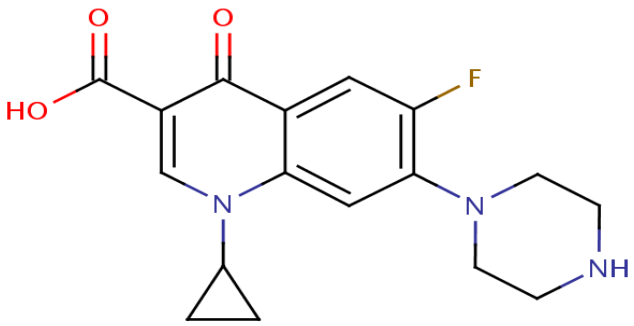
3.2.3 Analysis solutions of the used drugs

The solutions of the examined fluoroquinolones were prepared from the stock solutions. Ciprofloxacin and Moxifloxacin are available in the form of drip solutions in bottles kept under sterile conditions and away from light according to the recommendations of the manufacturer. Gatifloxacin is available in the form of powder.

3.2.3.1 Ciprofloxacin

1-cyclopropyl- 6-fluoro- 4-oxo- 7-piperazin- 1-yl- quinoline- 3-carboxylic acid

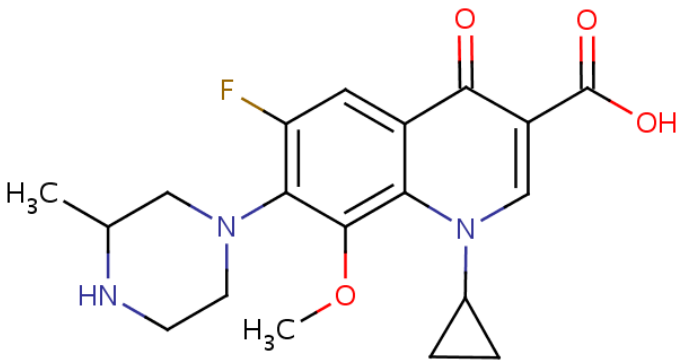
One bottle of 50 ml ciprofloxacin-drip solution (Ciprobay®, Bayer) was used as a stock solution. It contains 127.2 mg ciprofloxacin lactate and correspondingly, 100.0 mg ciprofloxacin (MW 331.35). Immediately before experiments, 100 or 500 µM solutions were prepared by diluting 833 µl or 4.16 ml, respectively from the bottle to make the final volume of 50 ml.



3.2.3.2 Gatifloxacin

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid

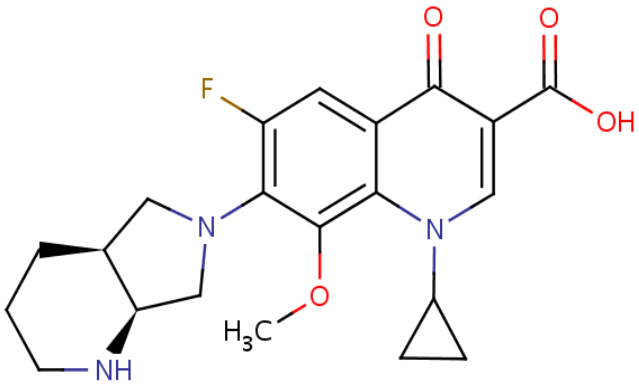
It is available as a pure substance (MW 375.4, Grünenthal GmbH, Aachen, Germany). To prepare 100 or 500 µM solution, 1.31 or 6.57 mg of gatifloxacin powder was dissolved in 35 ml of mKRB, respectively.



3.2.3.3 Moxifloxacin

1-cyclopropyl-7-[(1S, 6S)-2, 8-diazabicyclo [4.3.0] non-8-yl]-6-fluoro-8-methoxy-4-oxo- quinoline-3-carboxylic acid

It is available as an infusion solution (Avalox®, Bayer) in a 50 ml bottle containing 87.36 mg moxifloxacin hydrochloride (MW 401.44). The concentrations used for the measurements (100 and 500 µM) were made by mixing 1.37 or 6.85 ml in mKRB to reach the final volume of 40 ml, respectively.



3.2.4 Perifusion solutions of the ratiometric fluorescence microscopy

3.2.4.1 HEPES-buffered Krebs-Ringer-Bicarbonate solution (Krebs-Ringer-Basic medium)

For the perfusion experiments, mKRB medium was prepared by adding the below given volumes of stock solutions to give a volume of 1 L.

1. Stock solution 1 (0.90 % NaCl): 18.0 g sodium chloride (Merck) was dissolved in 2 L distilled water. 200 ml stock solution yield a final concentration of 115 mM NaCl.

2. Stock solution 2 (1.15 % KCl): 5.75 g potassium chloride (Merck) was dissolved in 500 ml distilled water. 8 ml stock solution yields a final concentration of 4.7 mM KCl

3. Stock solution 3 (1.62 % $CaCl_2 * 2H_2O$): 8.10 g calcium chloride (Merck) was dissolved in 500 ml distilled water. 6 ml stock solution yields a final concentration of 2.5 mM $CaCl_2$.

4. Stock solution 4 (2.1 % KH_2PO_4): 5.27 g potassium dihydrogen phosphate (Merck) was dissolved in 250 ml distilled water. 2 ml stock solution yields a final concentration of 1.2 mM KH_2PO_4

5. Stock solution 5 (3.82 % $MgSO_4 * 7H_2O$): 9.55 g Magnesium sulphate (Merck) was dissolved in 250 ml distilled water. 2 ml stock solution yields a final concentration of 1.2 mM $MgSO_4$.

6. Stock solution 6 (1.30 % $NaHCO_3$): 13 g sodium bicarbonate (Merck) was dissolved in a volume of 1000 ml distilled water and was then bubbled for a period of 30 min with CO_2. 34 ml stock solution yields a final concentration of 20 mM $NaHCO_3$

7. Stock solution 7 (6.5% fatty acid-free albumin, 7.74% HEPES, 154 mM NaOH): 32.5 g of fatty acid-free bovine serum albumin (Serva) was dissolved in 200 ml distilled water.

Then 38.7 mg HEPES (Sigma) was dissolved in 125 ml distilled water. To this solution 77 ml 1N NaOH (Merck) were added. Then, these two solutions were transferred in a 500 ml flask and it was filled up with distilled water to 500 ml. This solution was divided in 10-ml portions and stored at -20 °C. 8 ml stock solution yields a final concentration of 4.7 mM NaOH, 10 mM HEPES and 0.2 % Albumin.

For the microfluorometric measurements, pure HEPES solution was used instead of stock solution 7 to prevent formation of foams by albumin since the experiments require bubbling with Carbogen (mixture of 5 % CO_2 and 95 % O_2), for 20 - 25 minutes directly before and continuously during the experiments.

3.2.4.2 Carbonylcyanide-m-chlorophenylhydrazone (CCCP)

CCCP stock solution (20 mM): 4.09 mg of CCCP (Sigma, MW 204. 60) were dissolved in 1 ml DMSO. CCCP test medium (5 μM): 2.5 μl CCCP stock solution was added to 10 ml of a glucose-containing mKRB.

3.2.4.3 Sodium Azide (NaN_3)

NaN_3 stock solution (500 mM): 32.5 mg of NaN_3 were dissolved in 1 ml water.
NaN_3 test medium (5 mM): 100 μl of stock solution was added to 10 ml of mKRB.

3.2.4.4 D- 600 (Methoxyverapamil)

To prepare a 5 mM stock solution, 26 mg D 600 (Sigma, MW 521) was dissolved in a volume of 10 ml distilled water. Preparation of 50 μM D-600 solution was made by the dilution of 300 μl of the stock solution with 30 ml with mKRB.

3.2.4.5 Diazoxide

Stock solution (300 mM) was prepared by dissolving 69.21 mg of diazoxide (Sigma, MW 230.7) in 1 ml DMSO. Diazoxide test medium (300 μM): 100 μl of the stock solution was added to 100 ml mKRB.

3.2.4.6 Tolbutamide

Stock solution (50 mM) was prepared by dissolving 13.51 mg Tolbutamide (Serva, MW 270.3) in 1 ml 0.1N NaOH. Tolbutamide test medium (500 μM): 100 μl of the stock solution were added to 10 ml mKRB.

3.2.4.7 Potassium chloride solution

To prepare 40 mM KCl solution, 24.52 mg KCl (Merck, MW 74.56) was dissolved in 10 ml mKRB, which already contains an amount of 4.7 mM KCl.

3.2.4.8 Glucose-substituted (5 mM and 10 mM) Krebs-Ringer-Basic medium

D-glucose monohydrate (Merck MW 198.17) 99 mg, 198.2 mg and 396.4 mg were dissolved in a volume of 100 ml of mKRB, to obtain 5, 10 and 20 mM glucose concentrations in the perifusion medium, respectively.

3.2.5 Solutions of fluorescent indicator

3.2.5.1 Fura- 2/AM (Acetoxymethyl ester)

For the production of the 1 mM stock solution 50 µg Fura- 2/AM (Mobitec; Göttingen, MW 1001.86) was dissolved in a volume of 50 µl DMSO (Merck). The resulting solution was stored away from light at a temperature of 4 °C and was used for a maximum period of 3 weeks. To load the isolated β cells, 2 µl of the stock solution was mixed in an Eppendorf tube with 998 µl mKRB (see 3.2.4.1), so that a concentration of 2 µM Fura- 2/AM is achieved.

3.2.5.2 Fura-PE3/AM

Fura-PE3/AM stock solution (1 mM): 0.5 mg Fura-PE3/AM (Mobitec- Göttingen, MW 1258) was dissolved in 397 µl DMSO and divided as 50 µl stock solution in vials. Fura-PE3/AM incubation medium (2 µM): 2 µl of Fura-PE3/AM stock solution was dissolved in 998 µl of the glucose-containing mKRB.

3.2.5.3 Rhodamine 123

Rhodamine 123 (Sigma) was dissolved in DMSO at a concentration of 1 mg/ml and stored at 4 °C.

3.3 Experimental Animals

3.3.1 NMRI-albino mice

NMRI mice (12-14 weeks old, female) were used as donor animals for the isolation of the pancreatic islets and β cells (Harlan Winkelmann or Charles River). They were kept in Macrolon cages in air-conditioned rooms (25 °C) at a constant day/night cycle (12 -12) and they received standard pellet feed and water ad libitum. The NMRI albino mice were originally established in

1937 as a Swiss Out breeding mice at the National Institutes of Health and then were transferred to the **N**aval **M**edical **R**esearch **I**nstitute, hence the name.

3.3.2 SUR1 knock-out mice

In order to investigate the K_{ATP} channel-independent effects of fluoroquinolones, SUR1 knock-out mice were used. They were kindly provided by Lydia Aguilar-Bryan and Joseph Bryan (Pacific Northwest Research Institute, USA). Similar to the NMRI-albino, these mice were kept with 12 hours day and night rhythm at a temperature of 25 °C and received the standard food and drinking water ad libitum. Both strains were kept at Animal care facility, TU-Braunschweig. Principles of laboratory animal care were carried out in accordance with the standards established by the European Communities Council Directive.

3.4 Islets isolation and cell culture

3.4.1 Pancreatic islets isolation

The two most prevalent approaches for isolating islets from rodent pancreatic tissue differ, primarily, in the way collagenase enzyme was introduced to the pancreatic tissue. The primary objective of these methods is to provide a large number of tissue-free and intact functioning pancreatic islets as well as β cells (Ravier and Rutter, 2010).

1. The "conventional" digestion method (Moskalewski, 1965, Lacy and Kostianovsky, 1967; Lernmark, 1974) was based on cutting the pancreas into small pieces before the digestion with collagenase.

2. The injection method, in which the collagenase solution was injected into the bile duct to distend the pancreas via the pancreatic duct prior to excision of the pancreas from the sacrificed mouse (Gotoh et al., 1985; Zimny et al., 1993).

3.4.1.1 The ‘conventional’ method

First the mKRB and culture media should be prepared and the shaking water bath switched on to reach a temperature of 37 °C within 20 minutes. An NMRI mouse was sacrificed by cervical dislocation. The skin was disinfected with ethanol (70 %) and then the skin, the subcutaneous tissue and abdominal muscle were cut open. The pancreas was then completely excised and was transferred to a glass vessel containing mKRB (5 mM glucose). Before the beginning of the collagenase digestion, the tissue was made free from adjacent fat and then transferred in 2 ml mKRB solution to be cut with scissors into very small pieces for approx. 3 - 5 minutes. Then the tissue suspension was transferred to a glass vessel containing 1 mg collagenase. This glass

vessel was then shaken in a water bath and with a frequency of 328/min at 37 °C. The digestion was stopped just after 7 - 9 minutes (depending on the activity of the collagenase batch) by diluting the contents using mKRB and then shaking vigorously by hand (approx. 10 s). This suspension was left for about 5 minutes, sedimented to the base leaving the supernatant to be aspirated with a vacuum pump and the glass container was filled again with the glucose-containing mKRB. The remaining tissue was transferred to a black Petri dish for pancreatic islets to be freed from the surrounding exocrine tissue, under the stereomicroscope within about 30-40 minutes with the help of two milled needles. The islets appear spherical and white, approximately 50–250 μm in diameter. These features, particularly the white color of the islets in comparison to the relatively darker exocrine tissue, allow for rapid identification of the islets. An average of 60 islets per mouse could be obtained using this method.

3.4.1.2 Injection method

According to the injection method used by Gotoh et al. (1985) and Zimny et al. (1993), 1 mg collagenase was dissolved in 2 ml of mKRB medium. This solution was put in a 3-ml plastic syringe (Injekt, Braun), which lead this syringe with a sterile needle (BD Microlance) to be kept on ice. After sacrification, the mouse was then fixed on a clipboard with the caudal part showing away from the preparator. The peritoneal cavity was opened as described above. All the organs were placed on the left side to let the pancreatic duct visible. The punctum where the pancreatic duct joins the duodenum could be identified as a milky white area on the duodenum. The punctum was closed with a vascular clamp, which prevents the collagenase from escaping from the pancreas through the intestine. Under the stereomicroscope, the cooled collagenase was injected in the tense bile duct so that the solution passes retrograde via the pancreatic duct to fill the pancreas as shown in (Fig 3.1). The pancreas was separated carefully, without cutting it to prevent the collagenase from escaping. The intestine must not be cut, to avoid contamination.

The pancreas was removed using a sterilized forceps and was transferred into a 10-ml plastic Falcon tube (Sarstedt), which was kept on ice. This tube was then left for 8 - 9 minutes in a water bath at 37 °C to initiate digestion and then, with gentle shaking by hands for 60-90 seconds till the suspension looks homogenous with no or few clumps. Thereafter, digestion was then terminated with 5 ml of cold mKRB medium. Secondly, the digested pancreas was centrifuged for 15 seconds at 300 g (1340 rpm), the supernatant was removed, and the sediment was washed with mKRB medium and removed and centrifuged again. The digested pancreas was then filled with mKRB medium and transferred into a black dish.

For the production of single cells, the islets were collected (as described above) to be transferred in 5 ml of Ca^{2+} free solution for 10 minutes, during which the contents were vortexed twice for

one minute in the beginning and at the end, and then centrifuged at 1000 rpm for one minute. Then, under the clean bench, the supernatant was aspirated and the cells were re-suspended in RPMI 1640 containing 10 mM glucose, vortex for 10 seconds, centrifuged as above then the supernatant was removed leaving small volume which contains most of the β cells. The cells were incubated in a cell culture incubator 2 hours and half to allow the maximum numbers of cells in the centre of the plastic Petri dishes (Sarstedt) to attach to the cover slip before 2.5 ml RPMI 1640 medium with 5 mM glucose (3.2.2.6) was added for each Petri dish.

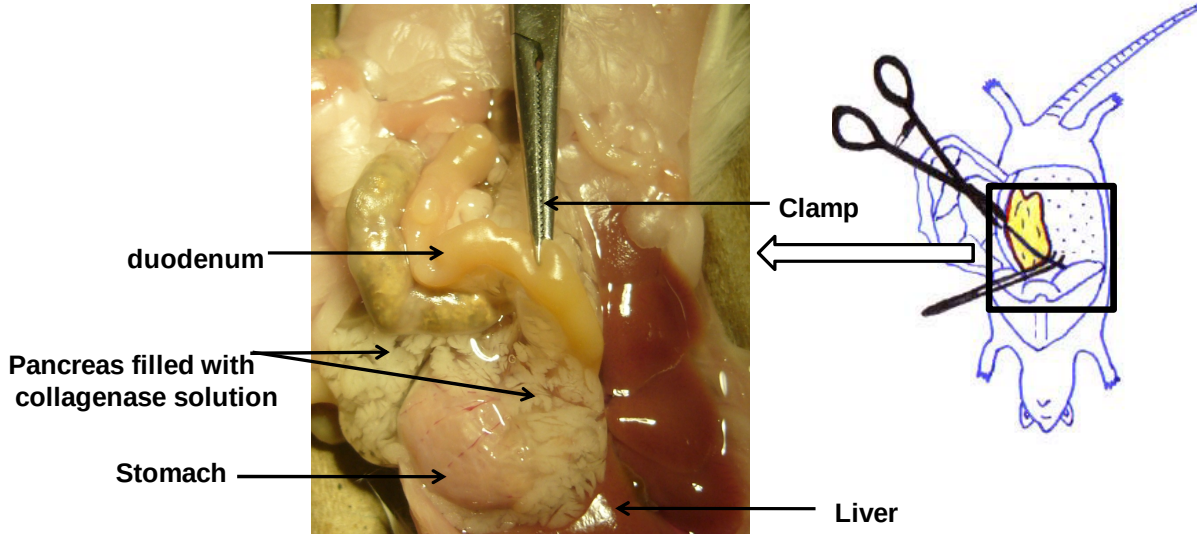


Figure 3.1 The injection method for isolation of the pancreatic islets from NMRI mouse. Scheme of collagenase injection into a mouse pancreas (Right). The pancreatic duct was clamped at the point where it joins the duodenum intestine. Then, 3 ml of collagenase was injected via the bile duct to fill the pancreas of NMRI mouse (Left).

3.4.2 Cultivation of isolated pancreatic islets

Under sterile conditions (clean bench), small Petri dishes were marked on the bottom by a circle to adjust the place for the cover glass, where collagen solution (Lenzen and Peckmann, 2001) was spread and left to dry for about 10 minutes. Then each a single pancreatic islet was transferred to each precoated cover glass in a volume of 30 µl RPMI-culture medium (containing 10 mM glucose). The islets were kept in the incubator for a period of 3 - 4 h. After this attachment period, 2.5 ml of the RPMI-medium containing 5 mM glucose was added (see chapter 3.2.2.6).

3.5 Microfluorometric measurement

3.5.1 Basic considerations for fluorescence measurements

Fluorescence involves the absorption of light to generate an excited state in a molecule which then emits light upon return to the ground state. This process is illustrated in the Fig 3.2A,

showing the major molecular pathways of a fluorophore in a Jablonski diagram. The lowest energy state, the ground state, is denoted by S0. This state is for most organic molecules an electronic singlet, in which all electrons have opposite spin. All chemical compounds absorb energy, which causes excitation of electrons bound in the molecule, such as increased vibrational energy or, under appropriate conditions, transitions between discrete electronic energy states (S1, S2, . . etc.). For a transition to occur, the absorbed energy must be equivalent to the difference between the initial electronic state and a high-energy state. This value is constant and characteristic of the molecular structure. This is termed the ***excitation wavelength***. If conditions permit, an excited molecule will return to ground state by emission of energy through heat and/or emission of energy quanta such as photons. The energy difference between the lowest energy peak of absorbance and the highest energy of emission is called the ***Stokes shift*** (Fig 3.2B). The fluorescent output is determined by the efficiency of light absorption and the amount of light emitted relative to what was absorbed (characterized by its ***quantum yield***).

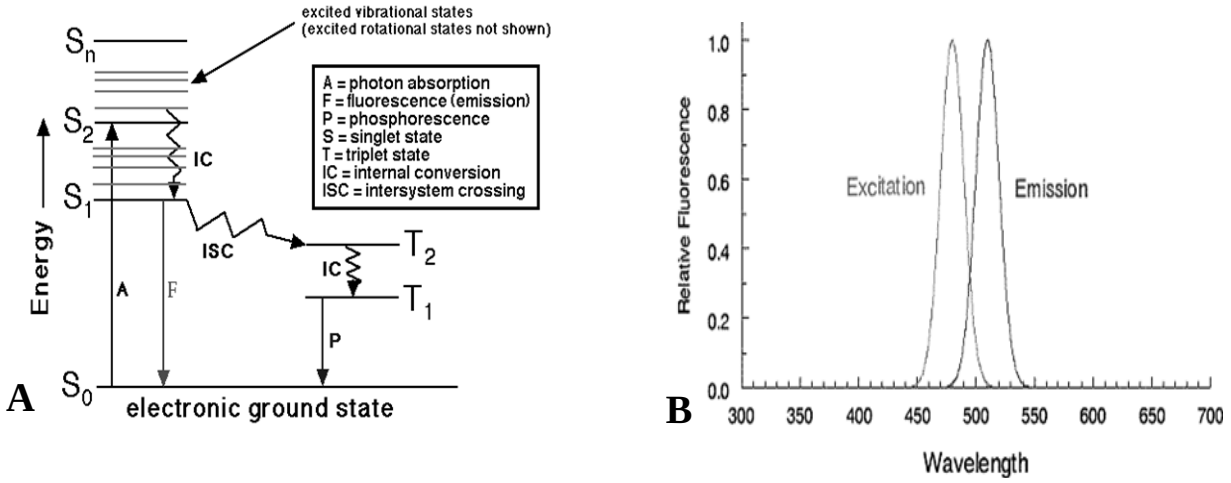


Figure 3.2 Jablonski diagram and Stokes shift. A) Jablonski diagram illustrating the major molecular pathways of a fluorophore upon absorption of a single photon. If the photon emission (shown in short wavelength blue in the diagram) occurs between states of the same spin state (e.g. S1 ---> S0) this is termed fluorescence. If the spin state of the initial and final energy levels are different (e.g. T1 --> S0), the emission is called phosphorescence (shown in longer wavelength red). **B)** Stokes shift is shown between the excitation and emission maxima (Wolf, 2007).

3.5.2 Principles of epifluorescence microscopy

Epifluorescence microscopy is a method of fluorescence microscopy in which both the excitation and emission light travel through the objective. Using a matched optical set that consists of an excitation filter, a dichroic beam splitter and a barrier filter, the epi-illuminator allows only light of the correct short, exciting wavelength to strike the preparation and only light of the longer, emitted wavelength to reach the eyepiece, detector or the camera (Fig. 3.3). In order to generate enough

excitation light intensity to furnish a level of emission capable of detection, powerful light sources are needed, usually arc (burner) lamps either xenon or mercury powered by a D.C. power supply. Xenon arc lamp is typically referenced using the registered trademark as **XBO** lamps (**X** for **Xe** or xenon; **B** is the symbol for luminance; **O** for unforced cooling). It contains in its emission spectrum, the excitation wavelengths of fluorophore Fura-2 and Rh123 and provides a largely continuous and uniform spectrum across the entire visible spectral region (300 - 650 nm).

To produce a dual wave length excitation, the following set up was employed:

An internal reflecting mirror is incorporated in the lamp house so that to produce a collimated light beam of high intensity. The lamp is coupled to two different quartz fibers light guide, which provide the necessary excitation of the indicator Fura-2 at two different wavelengths. These two fibers are electronically controlled by two shutters (Uniblitz shutter control unit with VMMD-1) with a programmable frequency to the excitation light. By this way, illumination will be blocked when data is not required and photobleaching could be avoided. The chosen wavelength (the monochromatic light) was then passed in the diffraction grating of quartz fibre light guide. By this way, light of a single wavelength was used each time the shutter opens (in case of Ca^{2+}, 340 and 380 nm).

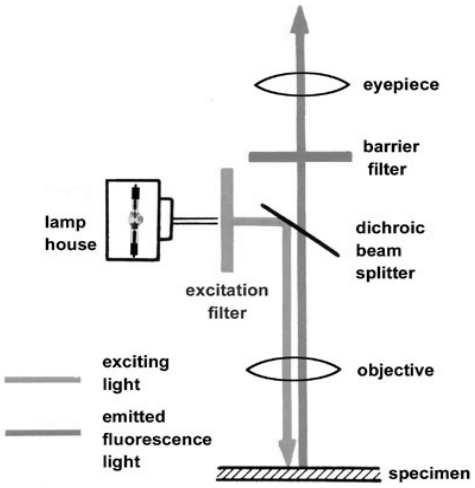


Figure 3.3 Illustration of the light path of the epifluorescence microscope. The light source used either high pressure mercury or xenon lamps. The production was made monochromatic light by excitation filter.

The dichroic mirror (also "chromatic beam splitter", abbreviated to CBS) is a key element of the fluorescence microscope and is used to separate the excitation and emission light paths. It reflects wavelengths of light below the transition wavelength value (into the objective) and transmits wavelengths above this value (to the eyepiece or detection system).

The dichroic mirror was mounted above the object at 45° in the light path and monochromatic excitation light (340 nm to 380 nm) is deflected by 90° in the direction of the object, which supported the separation between excitation and fluorescence light. This beam splitter reflects light of short-wave radiation (470 nm, i.e., the fluorescence excitation light) and allows longer-light (> 500 nm, i.e. the emission of fluorescent light) to pass, so that Fura-2 fluorescence light (maximum 510 nm) could pass through the dichroic mirror. The use of a single lens system provides an optical system focusing the excitation radiation to the object (condensation function) as well as the collection of the emitted fluorescence radiation. A Zeiss oil immersion lens (made of glass types of low autofluorescence) with 40 magnifications and a high numerical aperture (1.3) was used. It possesses high transmission in the near UV range (> 40 % at 340 nm) which makes it ideal for Fura loaded islets or cells.

As an imaging detector, a digital CCD (charge coupled devices) camera was used as the quantum yield of most fluorochromes is low. It captures the incoming photons of the fluorescence radiation and converts them into digital data (pixels). The camera can be adjusted to be synchronized with the changing excitation. The calculated cell fluorescence ratio (pixel-per-pixel division) was then converted into adequate picture elements, which constitute the regions of interest (ROI's). The kinetics was then calculated by using a special computer program (Fucal, TILL Photonics).

3.5.3 Measurement of the cytosolic Ca^{2+} concentration in perifused pancreatic islets

3.5.3.1 Properties of the ratiometric fluorescent indicator Fura- 2

The measurement of the free calcium concentration $[Ca^{2+}]_i$ in the cytoplasm of living cells with calcium-sensitive fluorescent dyes is a well-established technique (Roe et al., 1990; Moreton, 1994). Measurement of $[Ca^{2+}]_i$ in the pancreatic islets was carried out with 2λ procedures (measure the fluorescence of a dye after excitation with two different excitation wavelengths). It offers the ratiometric fluorescence measurements to be made so that the ratio (R) of two fluorescence intensities (F_{340}/ F_{380} X100) could be calculated. The fluorescence ratio is only dependent on the concentration of intracellular Ca^{2+} and independent of the indicator concentration. For 2λ process, indicator dyes undergo a change in their excitation spectra to other wavelengths “shift" upon Ca^{2+} binding. Fura-2/AM is established as a fluorescent dye suitable for $[Ca^{2+}]_i$ concentration (Grynkiewicz et al., 1985). For the measurements of $[Ca^{2+}]_i$ concentration, the calcium sensitive dyes, must have the following properties:

• Minimal interaction with the cellular processes.

• Good movement of the substances across the cell.

• The possibility of non-invasive measurement of Ca^{2+}-concentration

• High selectivity of binding to the Ca^{2+}-ions over other mono-and divalent ions.

Compared to the previously developed Ca^{2+} indicators, Fura-2 has a higher quantum yield of fluorescence and higher molar extinction coefficients (the ability to absorb photons). Therefore, a good signal-to-noise ratio (SNR) can be obtained at low probe concentration with much less disturbance of biological systems. Fura-2 exhibit K_d values that are close to typical basal Ca^{2+} levels in mammalian cells (~100 nM). Nevertheless, Ca^{2+} binding is prominently perturbed by physiological levels of Mg^{2+}; the K_d for Ca^{2+} of Fura-2 is ~135 nM in Mg^{2+}-free Ca^{2+} buffers and ~224 nM in the presence of 1 mM Mg^{2+} (Van den Bergh et al., 1995).

Esterification of the hydrophilic carboxyl group renders the dye cell permeable. Once within the cell, Fura- 2/AM (Acetoxy Methyl ester) is cleaved by the intracellular esterase enzymes freeing the negatively charged dye essentially trapping it within the cell where it is able to bind Ca^{2+} (Tsien, 1989). Sometimes AM ester hydrolysis is incomplete (Highsmith et al., 1986; Scanlon et al., 1987) or the fluorescence becomes compartmentalized within the organelles (Almers and Neher, 1985; Steinberg et al., 1987).

Fura-2 is a highly specific Ca^{2+} chelator and binding changes its fluorescent properties. In the presence of Ca^{2+}, maximum Fura- 2 fluorescence (at 510 nm emission) is observed at wave length 340 nm and in Ca^{2+} free conditions at 380 nm as shown in Fig 3.4. Therefore, it follows that the concentration of free intracellular Ca^{2+} is proportional to the ratio of fluorescence at F_{340}/F_{380}. Ca^{2+} binding shifts the excitation spectrum about 30 nm to shorter wavelengths, so that the ratio of intensities obtained from 340/380 nm or 350/385 nm excitation pairs is a good measure of $[Ca^{2+}]_i$; unperturbed by variable cell thickness or dye content (Fig 3.5). This "shift" is the reason that Fura-2 is one of the most common indicators for quantitative measurements of the $[Ca^{2+}]_i$ concentration (Lambert, 1999). The Grynkiewicz equation describe this relationship

$$[Ca^{2+}]_i \text{ (nm)} = K_d (R- R_{min}) / (R_{max}- R) S_{fb}$$

Where K_d is the Ca^{2+}-Fura-2 dissociation constant =225 nM, R= F_{340}/F_{380} ratio, R_{max} = F_{340}/F_{380} ratio under Ca^{2+} saturating conditions, R_{min} = 340/380 ratio under Ca^{2+} free conditions, and S_{fb} = ratio of baseline fluorescence (380 nm) under Ca^{2+} free and bound conditions.

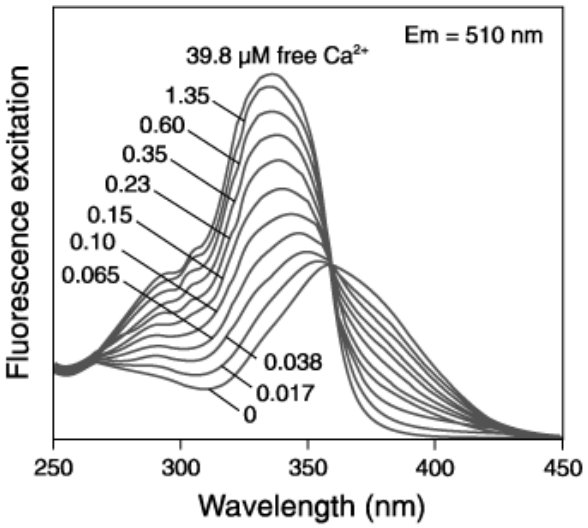
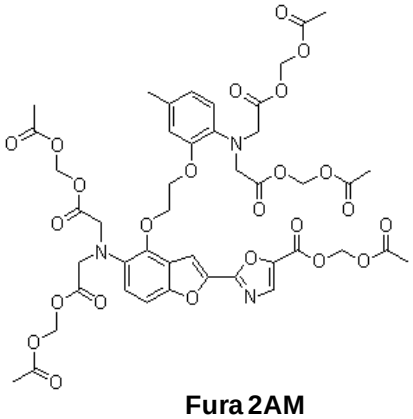


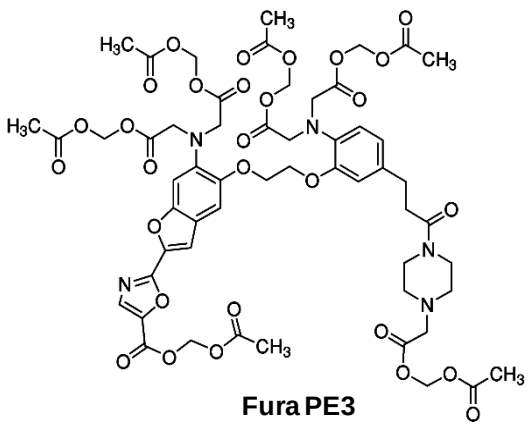
Figure 3.4 The Ca^{2+} dependent fluorescence excitation spectra of Fura-2 in solutions containing 0–39.8 µM free Ca^{2+}. Upon binding Ca^{2+}, fura-2 exhibits an absorption shift that can be observed by scanning the excitation spectrum between 300 and 400 nm, while monitoring the emission at ~ 510 nm (Mammano and Bortolozzi, 2010).

3.5.3.2 Properties of the ratiometric fluorescent indicator Fura- PE3

One of the most common problems seen with Fura-2 is its tendency to both leak out of cells and accumulate in organelles (Di Virgilio et al., 1990; Goligorsky et al., 1986). It is known to be released from the cells either passively or by anion transport (Jakob et al., 1998; Manzini and Schild, 2003). This leads not only to a decrease in the fluorescence emission but also to distortion of the results since the released indicator will report the Ca^{2+} of the extracellular space. For this reason, Fura-PE3/AM was designed by Dr. Poeni group (Austin, Texas) in 1995 to resist compartmentalization and leakage by virtue of an added positive charge containing a piperazine ring, the nitrogen with the adjacent carboxyl groups to form a Zwitterion (Vorndran et al., 1995). Fura-PE3 though shares the basic properties of Fura- 2, but with decreased rate of leakage which is estimated to be about 0.2 % per minute (Milner et al., 1998). This rate is slow enough for the cells to retain a useful amount of the indicator for several hours and makes it ideal for loading the islet cells (Vorndran et al., 1995).



Fura 2AM



Fura PE3

The concentration of fluorescent dyes in the incubation medium should be adjusted so that a sufficient dye level must be attained to achieve an adequate SNR. However, it was important to avoid high levels that may cause Ca^{2+} buffering by the dye itself and to protect β cells against the potentially toxic degradation products of hydrolysis (acetate and formaldehyde). On the other hand too low concentration will result in a very low fluorescence signal.

After cultivation period of 24-72 h, the pancreatic islets or islet cells were loaded just before the experiment. Certain criteria should be fulfilled in pancreatic islets, used for the experiments:

1. It had to be free from exocrine tissue
2. The pancreatic islets should be round and be enveloped by a thin capsule
3. They should preferably have a small diameter as both the degree of electrical coupling and calcium are inversely related to the islet size (Gilon et al., 1994; Ravier et al., 2002).

The perifusion chamber was specifically designed so that it had a depression in the middle to be filled first with mKRB buffer. Laboratory vacuum grease (Glisseal®) was used as an adhesive and sealing compound on the cover glass. After loading the islet or islet cells attached to the cover slip with the respective indicator, it was placed to be firmly fixed in the middle the perifusion chamber in such a way that the solutions flowing around it, avoiding the possible entrance of air bubbles. With the help of the downstream peristaltic pump, an even flow through the system could be ensured (the flow speed of 200 µl / min). After applying a drop of immersion oil (Leitz, Wetzlar) with low fluorescence, the islet or cells were focused in the transmitted light. To avoid any interference with the physiological functioning of the pancreatic islets, the temperature was kept at 36 °C during the experiments (Nadal et al., 1994). In addition, the perifusion solutions were heated using a water bath and the lens was wrapped with a current-carrying resistor foil and thereby heated (not applied on single cells). The chamber and the objective were connected through the immersion oil to achieve a temperature of 36 °C in the perifusion chamber. The water bath containing the test solutions was adjusted at 44 - 46 °C and the resistance film on the lens was heated to 45-50 °C. When these conditions were fulfilled, the measured temperature in the perifusion chamber was ~ 36 °C. For the fluorescence measurement, the laboratory was kept dark in order to minimize measurement errors due to ambient light. After completion of the experiment the chamber as well as the tubes were cleaned with distilled water and dried.

Ratio images were obtained by acquiring pairs of images at alternate excitation wavelengths: one image for 340 nm and the other for 380 nm excitation wavelengths. The advantage of this system is that it enables to select images stored in the different regions (ROI's) to be compared as shown in Fig 3.5. In addition, for data analysis and calculation of kinetics, the pancreatic islet

had been divided into 6 sections, a ROI in the centre, and the other ROI's were positioned peripherally in a clockwise direction (Ravier et al., 2002). For testing the functional integrity of the cultured pancreatic islets, they were exposed to 10 mM glucose to show the oscillations and their synchronicity throughout the islet (Fig 3.5).

3.5.3.3 Experimental procedure to achieve a net Fura fluorescence ratio in the presence of non- negligible interference

The measurements of $[Ca^{2+}]_i$ concentration were rendered difficult by the autofluorescence of the fluoroquinolones which increased values of the Fura-fluorescence ratio (Gross Fura fluorescence ratio). Therefore, for each individual compound, a correction procedure was necessary. This was achieved by repeating each experiment with sham-loaded pancreatic islets or β cells i.e. the islets or the islet cells were incubated with mKRB containing 5 mM glucose without Fura. The fluorescence trace excited at 340 nm and that excited at 380 nm were subtracted from the corresponding trace obtained with Fura-loaded islets or β cells (Fig 3.6).

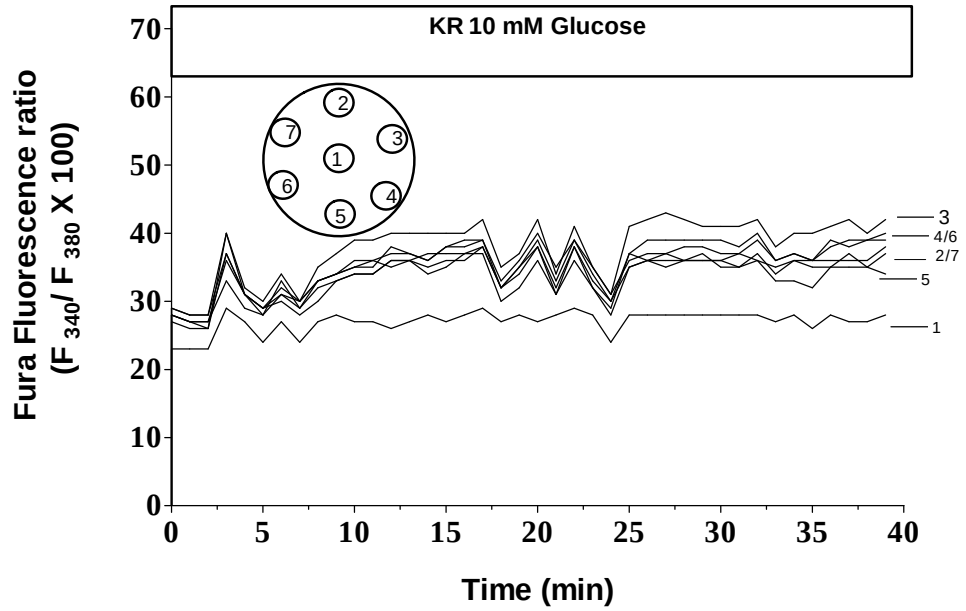


Figure 3.5 Determination of $[Ca^{2+}]_i$ concentration in the presence of Krebs-Ringer medium containing moderate stimulatory level of glucose (10 mM). A cultured pancreatic islet was loaded with Fura-2 AM. The islet show oscillatory pattern. Selected regions of interest (ROI_s) are numbered beginning from the islet core (sub-region 1) then followed by the peripheries (sub-regions 2-7).

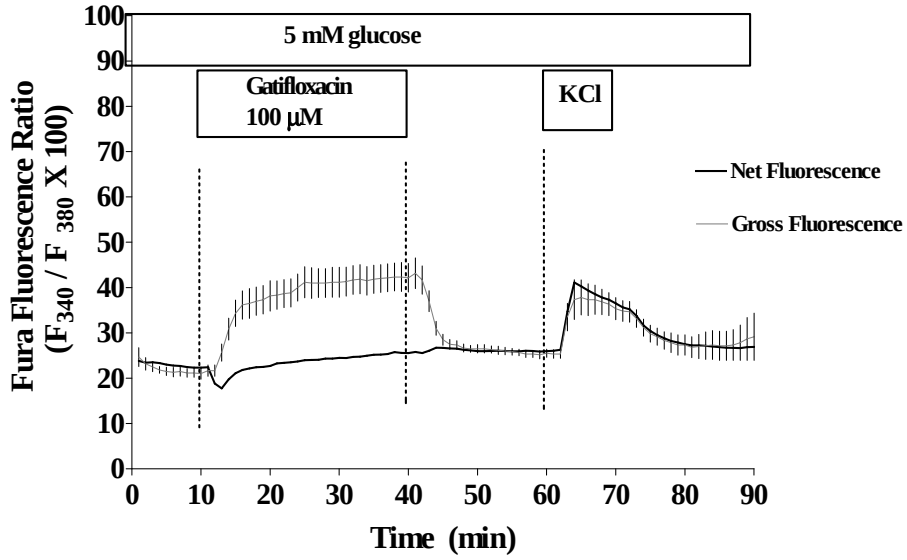


Figure 3.6 Difference between the gross fluorescence of gatifloxacin and the Ca^{2+}-dependent Fura-fluorescence ratio .The gross fluorescence represented by the grey line is the uncorrected ratio between the excitation wave lengths of 340 nm and 380 nm. The black line represents the net Fura fluorescence ratio after subtraction of the endogenous fluorescence of gatifloxacin at both wavelengths i.e. the pancreatic islets as well as β cells are incubated with Krebs Ringer medium containing 5 mM without Fura (Sham-loaded).

3.5.4 Measurement of NAD(P)H autofluorescence

The characteristic autofluorescence of NADH (and also NADPH) enables a view on mitochondrial function and cellular energy metabolism (Chance and Baltscheffsky, 1958). The term autofluorescence is used to distinguish it from the fluorescence of indicators that are artificially introduced. It is important to emphasize that these changes in signal do not indicate the net changes in the absolute size of the total pool but rather a change in the balance of reduced to oxidized form (Duchen et al., 2003).

The autofluorescence signal measured described in this protocol stems from both mitochondrial and cytosolic NADH and NADPH. As the autofluorescence spectra are identical (Fig 3.7), it is not possible to distinguish between the signals originating from these two; for this reason NAD(P)H, will be used to indicate that the signals are derived from either NADH or NADPH, or both (Schuchmann et al., 2001). Several factors suggest that the bulk of signal originates from the mitochondrial pool of NAD(P)H not only in pancreatic islets or β cells but also in other cell type in which mitochondria represent a substantial proportion of the total cell volume (Patterson, et al., 2000). One of the properties that strengthen the mitochondrial component of the signal is that binding of NADH to membranes enhances the fluorescence, whereas enzymatic binding tends to quench the cytosolic fraction. An increase in the activity of Krebs cycle, through the Ca^{2+} dependent up-regulation of the enzymes or through increased delivery of substrate will

increase the balance in the favour of NAD(P)H (Mojet et al., 2001). For the determination of NAD(P)H concentration in the pancreatic islets, single wave length approach had been used. The excitation and emission spectra of NAD(P)H molecules are relatively broad (peak excitation in the near UV ~ 340-360 nm; emission in the blue range ~ 430–450 nm) as shown in Fig 3.7A. High pressure mercury lamp (Heraeus-Hanau, ST41) was chosen as a light source for specific stimulation of NAD(P)H molecules. This mercury lamp emits light in the range between 360-370 nm, i.e. in the area of the absorption maximum of the fluorophore sufficient for excitation of NAD(P)H molecules.

The Excitation light was directed using Leitz epifluorescence microscope which differs in some aspects compared to that used for calcium measurements.

1. A Uniblitz-shutter (Vincent, Rochester, USA) operated at a frequency of 1 Hz with an exposure time of 0.2 s, which close the light in order to avoid photobleaching.
2. An Excitation filter: Band Pass 365 $\pm$ 15 nm, WB 50 (Omega Optical, Brattleboro, VT).
3. Emission filter: 450 ± 32 nm (Omega Optical, Brattleboro, VT).
4. The emission was separated by a dichroic mirror at 405 nm.
5. The fluorescence was recorded by a photon-counting multiplier (Hamamatsu Photonics, Japan; H-3460-04). The resulting ECL pulses were transformed into TTL pulses, which could then be counted and displayed to the computer via a Counter/Timer Board (CTM-05) (Plug-in electronic, Eichenau, Germany). The data acquisition program used was Labtec Notebook (Labtec Inc, Wilmington, Massachusetts, USA). The photomultiplier provides the possibility of detecting very low intensities with a high resolution.

For measurement of NAD(P)H autofluorescence, the islet (attached to the cover slip) was inserted in a purpose-made perifusion chamber on the stage of an epifluorescence microscope equipped with a mercury arc lamp and a temperature-controlled objective (Fluar 40x, 1.3 N.A., Carl Zeiss, Göttingen, Germany).

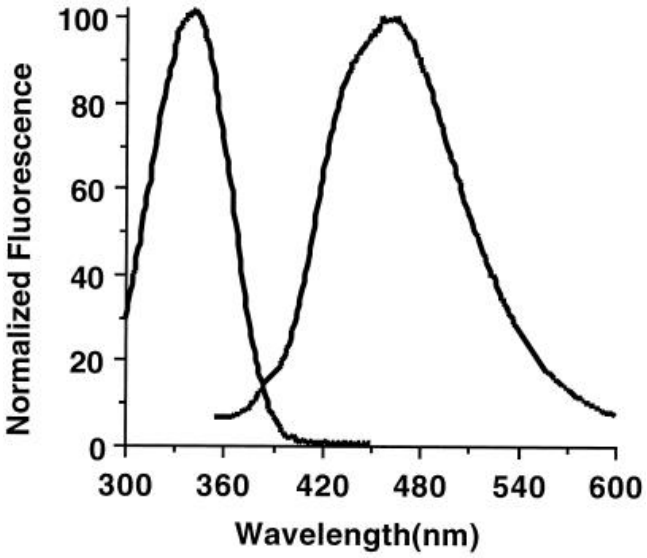


Figure 3.7 NAD(P)H excitation and emission spectra. Purified NADH and NADPH were suspended to 1 µM concentration in 50 mM Na_2HPO_4/50 mM sodium acetate/50 mM glycine, pH 7.0. The excitation spectrum was obtained by exciting at wavelengths from 300 nm to 460 nm while detecting emission at 465 nm. The emission spectrum was obtained by exciting 340 nm and scanning the emission wavelength from 350 nm to 600 nm. NADPH spectra exactly overlay NADH spectra (Patterson et al, 2000).

3.5.5 Measurement of FAD autofluorescence

Another source of redox related autofluorescence comes from oxidized cellular flavoproteins (Panten and Ishida, 1975; Chance et al., 1979). Like NAD(P)H, Flavin Adenine Dinucleotide (FAD) show differential fluorescence between their oxidized and reduced states. In contrast to NAD(P)H, these molecules are fluorescent in their oxidized state as their electrons assume maximum resonance, becoming prone to brief excitation to a higher energy state by blue light, followed by reversion to lower energy orbitals with the emission of green light. FAD are excited with 440 ± 10 nm excitation, away from the maximum excitations of tryptophan (~ 300 nm) and porphoryins (~ 400 nm) (Wagnières et al., 1998). The emission separated by a dichroic mirror at 455 nm and filtered by a 502 nm long pass filter (Omega Optical, Brattleboro, VT) (Fig 3.8). FAD molecules have the particular advantages of being spatially restricted to mitochondria, and thus being a direct measure of cellular metabolism (Husson et al., 2007).

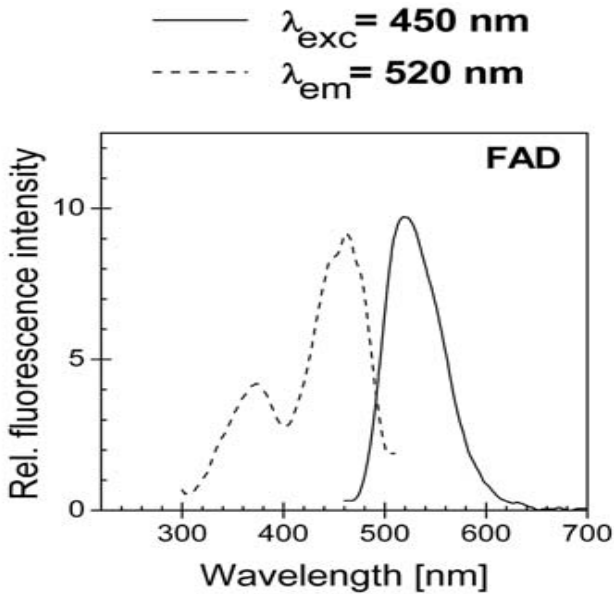


Figure 3.8 Fluorescence excitation and emission spectra of standard FAD. Excitation spectrum (broken line) was taken at 520 nm emission, emission spectrum (solid line) with excitation at 450 nm **(**Munro and Noble, 1999)

3.5.6 Measurements of the Rhodamine 123 fluorescence as a parameter of ΔΨm in perifused single pancreatic β cells

One full turn of the Krebs cycle generates 3 NADH and one $FADH_2$ molecules, which convey reducing equivalents to the respiratory chain. The increased supply of reducing equivalents is expected to increase the rate of electron transport which should increase the proton efflux from the matrix space. During the redox reactions, these protons are translocated across the inner mitochondrial membrane to the intermembrane space, generating an electrochemical proton gradient that is expressed largely as a membrane potential of the order of 150 - 200 mV (negative inside i.e. in the matrix space). This potential is referred to as “delta psi"(ΔΨm) and provides the driving force for proton influx through the F_1F_0 - ATP synthase. Finally, ATP is generated and transported to the cytosol (Fig 3.9) (Mitchell and Moyle, 1967).

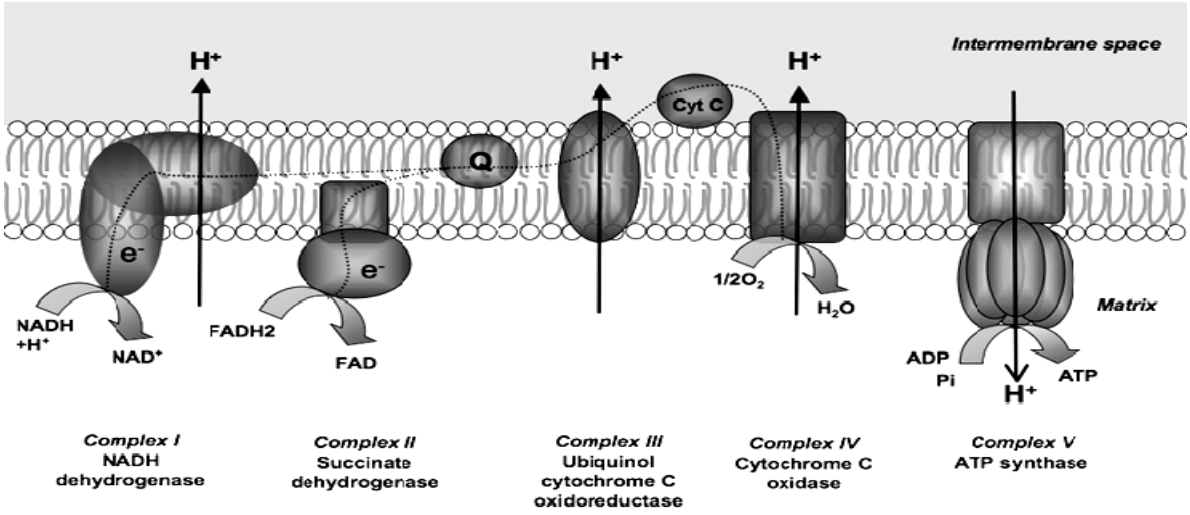
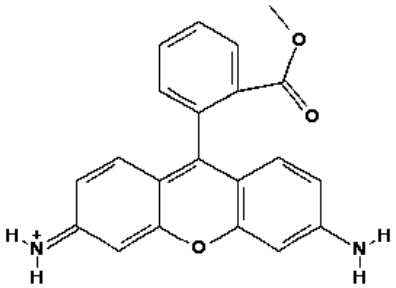


Figure 3.9 Mitochondrial respiratory chains. It consists of four enzyme complexes (complexes I - IV) and two intermediary substrates (coenzyme Q and cytochrome c). The $NADH+H^+$ and $FADH_2$ produced by the intermediate metabolism are oxidized further by the mitochondrial respiratory chain to establish an electrochemical gradient of protons, which is finally used by the F1F0-ATP synthase (complex V) to produce ATP (modified from Bellance et al., 2009)

Rhodamine 123 (Rh123 as shown below) is a fluorescent lipophilic cationic dye known to be specifically partitioned into negatively charged mitochondrial membranes. It has an excitation spectrum with the maximum of 500 nm and a molar extinction coefficient of 7.5 x 10^4 M^{-1} cm^{-1} in water (Darzynkiewicz et a1., 1981). Previous studies using Rhodamine 123 to measure mitochondrial activity established that ($\Delta\Psi m$) hyperpolarize in response to increased glucose concentration in β cells leading to redistribution within the cell. The increased mitochondrial Rh123 uptake and concentration lead to self-quenching of fluorescence within the mitochondria and to a decrease of the total signal (Nunemaker et al., 2004; Kindmark et al., 2001; Krippeit-Drews et al., 2000). In response to mitochondrial depolarization, the dye will leave mitochondria and move into the cytosol resulting in an increase of the total fluorescence signal. Mitochondrial poisons (uncouple respiration from phosphorylation) that dissipate the mitochondrial membrane potential, such as CCCP increase Rh123 fluorescence by preventing its uptake (Rustenbeck et al., 1997a; Anello et al., 2005). Similarly, electron transport inhibitors such as azides (e.g. NaN_3) eliminate Rh123 uptake.



Rhodamine 123

For measurements of ($\Delta\Psi m$), pancreatic β cells attached to the cover slip were loaded by 10 μg/ml of Rh123 for 10-15 min at 37 °C. Thereafter, the cells were washed and transferred to the perifusion chamber. The set up of epifluorescence microscope is similar to that used for calcium measurements except some features that were changed to fulfil the fluorescence characters of Rh123 being excited at 490 nm and its emission was collected at 535 nm. Excitation was performed by xenon lamp at 495 nm using an excitation filter (BP 450–490 nm). The emitted fluorescence was passed through a 500-nm long-pass dichroic mirror and a 535/25 nm band-pass filter (filter block I3, Leica).

3.6 Measurement of the adenine nucleotide content in pancreatic islets

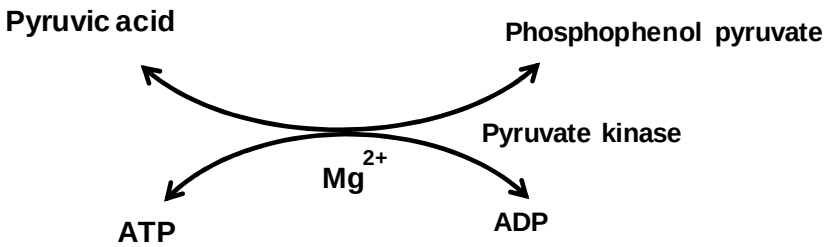
3.6.1 Principle of ATP/ADP measurement

ATP is detectable in all metabolically active cells and its concentration decreases rapidly when cells lose their vitality and die (Kangas et al., 1984). At present, there are no direct fluorescent indicators for ATP or ADP. Rather, ATP is measured by a luminescence reaction where the emitted light is proportional to the ATP concentration (Mojet et al., 2001).

The determination of ATP and ADP content in the isolated mouse pancreatic islets was done by a modification of the procedure used by Detimary et al. (1996). For the measurement of ATP, a bioluminescence kit of Sigma was used. When light emission was initiated by the addition of luciferase into a reaction mixture containing Mg^{2+} (or another divalent cation such as Mn^{2+}), and luciferin (4,5-dihydro-2-6-hydroxy-2-benzothiazolyl thiazole-4-carboxylic acid), where all components are near or at saturating concentrations, the luminescence observed is proportional to the ATP content in the samples. The ADP content was indirectly determined by prior conversion of ADP to ATP by pyruvate kinase (PK) reaction. The latter catalyzes the formation of ATP and pyruvate from ADP and phosphoenol pyruvate. Seliger and McElroy (1960) describe the reaction as follows:

$$\text{Luciferin + ATP} \xleftrightarrow{\text{Luciferase}} \text{Luciferyl-AMP+ } PP_i$$

$$\text{Luciferyl-AMP+}O_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin + AMP + } CO_2 \text{ + Light}$$



The transition of the Oxyluciferin is associated with light emission of the wave length 562 – 560 nm (green yellow light) which is directly proportional to the ATP concentration. Because the quantum yield of this bioluminescence reaction is 0.88, almost one photon is emitted per reaction cycle. The multiple photon counter used to measure ATP luminescence, can detect even single photons with high sensitivity.

3.6.2 Incubation and sample preparation

The sample and control were incubated simultaneously. To prepare the incubation, 10- ml Eppendorf tubes were filled with 1.5 ml distilled water had been saturated with Carbogen for 5 minutes in a water bath at 37 °C. Then 15 freshly isolated pancreatic islets were transferred in 50 µl mKRB into 1.5 ml Eppendorf tube containing 450 µl test solution. The Eppendorf tube containing the islets was placed inside the 10 ml Falcon tube and this assembly was again placed under a stream of Carbogen for about 5 minutes. Thereafter, the outer tube was tightly closed and was placed in a shaking water bath (shaking position 7, Kötterman) at 37 °C. The incubation conditions were made to mimic the experimental protocol used with the reducing equivalents. After 60 minutes pre-incubation under the above conditions, the test agents (glucose or fluoroquinolones) were added in a volume of 500 µl (pre-warmed and equilibrated with Carbogen) , resulting in a total incubation volume of 1000 µl. After a short vortex-mixing, the incubation was continued for the specified time. At the end of incubation time, the Eppendorf tubes were removed and placed on ice. 800 µl of the incubation volume was removed and 100 µl of ice-cold 15 % trichloroacetic acid (TCA) was added to the remaining volume of 200 µl containing the islets to precipitate the proteins. After vortex mixing for 1 minute, the cups were placed on ice and the proteins were sedimented by centrifugation (10.000 g at 4 °C for 3 minutes). To eliminate TCA from the supernatant, 240 µl of supernatant was mixed with 900 µl of water- saturated diethyl ether in Eppendorf tube, and the ether phase containing TCA was removed and discarded. This procedure was repeated 3 times to ensure complete removal of TCA. Thereafter, the samples were mixed with 240 µl neutralization buffer (20 mM HEPES + 3 mM $MgCl_2$ were dissolved in water), the pH of which was adjusted to 7.75 with 1N KOH. After this treatment, the samples could be stored at -18 °C or processed further.

3.6.3 ATP/ADP measurement

For the measurements of ATP content, 50 µl (from a total volume of 480 µl) was added to 300 µl dilution buffer. 100 µl of this mixture was pipetted into a 96-well plate. The micro-well plate was inserted into the luminometer (Victor^2 multilabel counter, Wallace, PerkinElmer) and the following steps were done automatically by the measurement program of the luminometer. 100 µl assay mix (dissolved 1:50 with dilution buffer) was injected into the well in the measurement

position. After shaking and a delay time of 10 s, the resulting luminescence was measured with a photon counting multiplier. The raw data was thus expressed as counts per second.

The measurement of ADP required the previous conversion of the ADP to ATP. To this end, 50 µl (from the above mentioned 480 µl sample) was mixed with 300 µl dilution buffer, containing additionally phosphoenol pyruvate (1.5 mM, Boehringer) and pyruvate kinase (2.3U/ml, Boehringer). After incubation for 45 min at 37 °C, 100 µl of this mixture was pipetted into a 96-well plate and measured as described for the ATP content.

3.6.4 Preparation of the ATP and ADP standard curves

The lyophilized powder of the assay mix (ATP bioluminescence kit, Sigma) was dissolved in 5 ml distilled water and stored in aliquots at - 20 °C. For the experiments the assay mixture was diluted in the ratio 1: 35 with assay mix dilution buffer (also contained in the sigma kit). Based on the concentration of $2x10^{-7}$ pmol ATP, the stock solutions were diluted so that the resulting standard curve comprised values from of 0-10 pmol ATP. The dilution was done with sterilized ultrapure water. For the luciferase reaction, 5 µl of each standard dilution were mixed again with 345 µl of mKRB plus dilution buffer. The composition of the resulting mixture mimics exactly the mixture of the biological samples plus reaction buffer. 100 µl were transferred per well of the microliter plate.

The various dilutions of ADP standard curve were first incubated for 45 minutes at 37 °C to achieve an enzymatic conversion of the ADP to ATP. To this end, 5 µl of each standard dilution was first diluted to 50 µl and then treated in the same way as described above for the biological samples.

3.6.5 Luminescence measurement and data analysis

For better precision, the average values of two measurements were calculated. Both ATP and ADP standard curves could be fitted with a linear function, which permits the use of linear regression to calculate the adenine nucleotide content from the luminometric signal. The ADP-content was calculated as the difference between the ATP in a sample and the ATP content after enzymatic conversion, which corresponds to the sum of ATP plus ADP. Thus the final result of this measuring procedure is the ATP and ADP content per islet. For a more sensitive detection of significant differences, the calculated ATP- to ADP- ratios were normalized to 100 % for each single control experiment.

3.7 Electron microscopic examination

The pancreatic islets were extracted as described in 3.4.1.2. Batches of 50 islets were pooled and cultured with RPMI medium using two different concentrations for each of the 3 drugs; 10 and 100 μM and without addition of penicillin/ streptomycin to avoid any possible interaction. After tissue culture for 20 hours, the islets were fixed for electron microscopy (Jörns et al., 1997) by immersion in a solution containing para-formaldehyde (2%) and di-glutaraldehyde (2%) in cacodylate buffer (0.1 M, pH 7.3). The tissue was post-fixed in 1% osmium tetroxide (OsO_4) for 1 h and embedded in epoxy resin. Thin sections of the islet pellets were cut at 50 nm with an ultramicrotome (Ultracut, Reichert-Jung, Nussloch, Germany), placed on nickel grids and contrast-stained with uranyl acetate and lead citrate. The thin sections were viewed in an electron microscope EM 9 (Zeiss, Germany). In initial qualitative studies, 10–15 islets of different sizes in each pellet were examined. Exocrine parenchymal cells and α, β, δ were identified on the basis of their morphology and the incidence of degenerative changes induced by the test compounds in each of the cell types was observed.

4. Results

4.1 Effect of fluoroquinolones on $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ concentration of pancreatic islets was analyzed in order to gain insight into the insulinotropic effect of the fluoroquinolones. Changes in $[Ca^{2+}]_i$ were measured in pancreatic islets as well as β cells of female NMRI mice using the fluoroprobe Fura-PE3 and Fura-2/AM, respectively. The results of the ratiometric Fura-2 based fluorescence measurements presented for each fluoroquinolone. The uncorrected data was defined as Fura- Gross fluorescence ratio, while the corrected Ca^{2+} - dependent fluorescence signals was corresponding to the net Fura fluorescence ratio. As each of the three used compounds had its own endogenous emission fluorescence and the corresponding ratio, the dissociation between the fluoroquinolone-specific autofluorescence and Ca^{2+}- dependent Fura fluorescence ratio was based on the principles outlined in Chapter 3.5.3.3.

The functional integrity of the cultured β cells together with pancreatic islets was tested by exposure to a maximum depolarizing K^+ concentration (40 mM). This stimulus evoked increase in $[Ca^{2+}]_i$ regardless of the glucose concentration (Gilon and Henquin, 1992; Rustenbeck, 1999). At the same time, the extent of K^+-induced depolarization was used as an internal standard for the assessment of $[Ca^{2+}]_i$ -modulating potency of the tested fluoroquinolones. The control phase was carried out initially from minute 10 - 20 prior to the actual exposure of the respective test drug. In the view of the variability among individual islets/ β cells, six islets from two different NMRI albino mice was used for the calculation of mean ± SEM of Fura fluorescence ratio. During the first 10 minutes, Ca^{2+} signal profile was initially recorded, with two different glucose concentrations; basal glucose concentration (5 mM) and a moderately effective glucose stimulus (10 mM).

4.1.1 Glucose-dependence effect of fluoroquinolone induced $[Ca^{2+}]_i$ changes

The effect of 100 μM gatifloxacin on the $[Ca^{2+}]_i$ was assessed in the presence of basal glucose. The stimulation of cultured islets with an exogenous K^+ concentration (40 mM) over a period of 10 minutes caused an immediate increase in the Fura fluorescence ratio. The maximum level of depolarization (minute 13) corresponded to a fluorescence ratio of 86 units. This was followed by a plateau phase. Restoration of the fluorescence ratio nearly to the basal level (54 units) occurred when K^+ was washed out. Exposure of the islets to gatifloxacin from minute 30- 50 did not produce any significant increase in the net ratio compared to the pre-stimulatory ratio. Perifusion of the islets with KCl together with gatifloxacin from minute 50-60 was still capable of inducing the characteristic increase in the ratio to 67 units; though not to the same extent as

the first exposure to KCl, but still effective (Fig 4.1A). An alternative protocol was used in which the perifusion with gatifloxacin from minute 10 - 40 could not produce any significant increase in the net ratio. When perifused with KCl from minute 60 - 70, the islets responded well and the ratio was increased from 24 to 41 units (Fig 4.1B).

In the next protocol, the islets were perifused with 100 µM gatifloxacin from minute 10 - 30, followed by increase to 500 µM from minute 30 - 50. As shown before, gatifloxacin did not cause a significant increase in $[Ca^{2+}]_i$ at 100 µM, but 500 µM produced a clear cut increase by 28 units. Gatifloxacin washout resulted in immediate re-establishment of the fluorescence ratio even to a level below pre-stimulatory values then returned rapidly to reach a steady state. The use of D-600 from minute 70 - 90 slightly reduced the ratio (Fig 4.2).

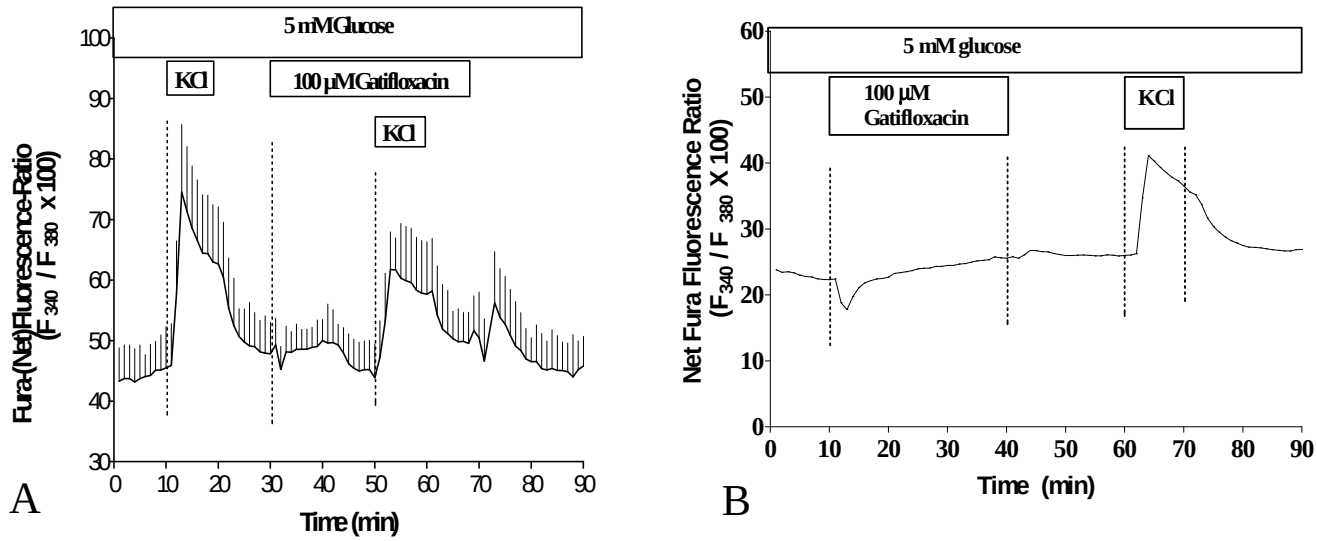


Figure 4.1 Effect of gatifloxacin on $[Ca^{2+}]_i$ in the presence of a basal glucose concentration in NMRI mouse islets. A) Pancreatic islets have been incubated for a period of 45 minutes with Fura-2. During the entire experimental period of 90 minutes a Krebs-Ringer medium with basal glucose concentration of 5 mM was used. The functional integrity of the islets was tested from minute 10-20 by the characteristic response to a maximum K^+-depolarizing stimulus. The islets were then exposed to gatifloxacin 100 µM from minute 30-50 where no increase in $[Ca^{2+}]_i$ was observed. The islets were even still responsive to KCl in the presence of gatifloxacin. **B)** The islets were first exposed to gatifloxacin from minute 10-40, again show no influence on the $[Ca^{2+}]_i$. The islets still respond to KCl after pre-exposure to gatifloxacin.

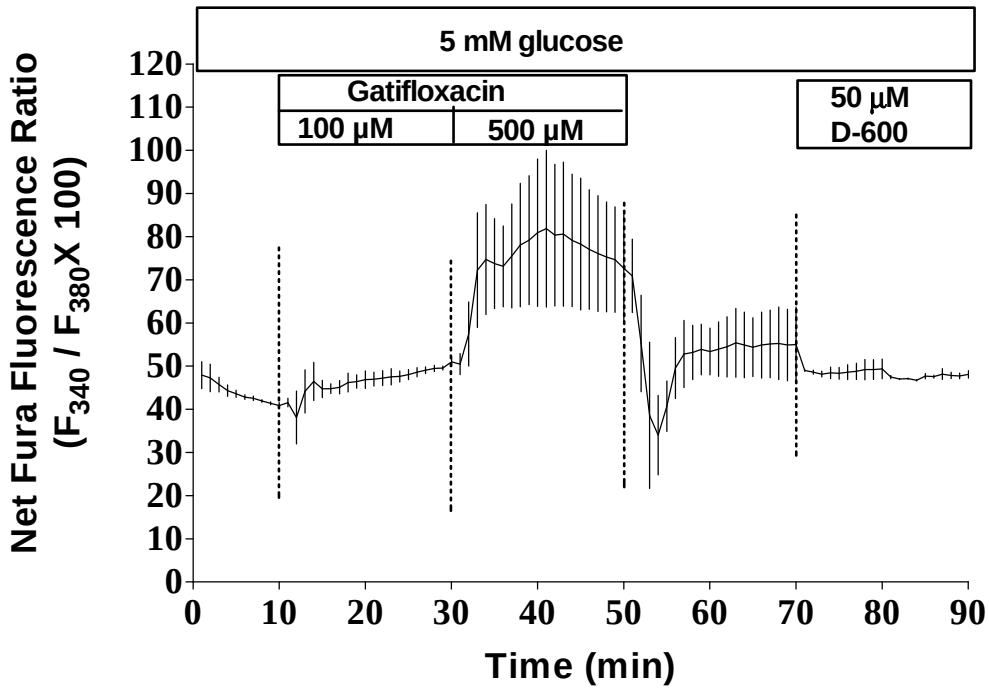


Figure 4.2 Effect of two different concentrations of gatifloxacin on $[Ca^{2+}]_i$ in the presence of a basal glucose concentration in NMRI mouse islets.. Cultured normal mouse β cells were perifused with Krebs-Ringer medium containing 5 mM glucose. From minute 10-30, the medium contained 100 µM gatifloxacin and then increased to 500 µM from minute 30-50. After wash-out the basal value of the net Fura fluorescence was established by 50 µM D600. Values are means ± S.E.M of 26 loaded and 30 sham-loaded β cells from 4 experiments each.

The effect of 100 µM gatifloxacin was then assessed in the presence of 10 mM glucose concentration. Perifusion with gatifloxacin from minute 40 - 70 induced a pronounced increase in $[Ca^{2+}]_i$ with latency of 2 minutes, and then a steady state was reached till the end of perifusion (Fig 4.3A). The magnitude of the effect was higher than that of a preceding KCl depolarization. The dependence of Ca^{2+} influx through calcium channels as a result of closure of the K_{ATP} channels was also analyzed. This was done by using diazoxide and D-600, respectively. First, gatifloxacin caused increase in $[Ca^{2+}]_i$ after a 3 minutes latency period to reach a steady state of 39 units. Then, 300 µM diazoxide from minute 50 - 70, caused a partial reduction of fluorescence ratio to a constant level of 33 units. Addition of 50 µM D-600 reduced the mean values somewhat, but did not re-establish the pre-stimulatory values. The typical effective increase in Fura fluorescence ratio produced by KCl depolarization was from 25 to 37 units. Stoppage of KCl perifusion (at minute 20) reduced the fluorescence ratio to pre-stimulatory values (Fig 4.3B).

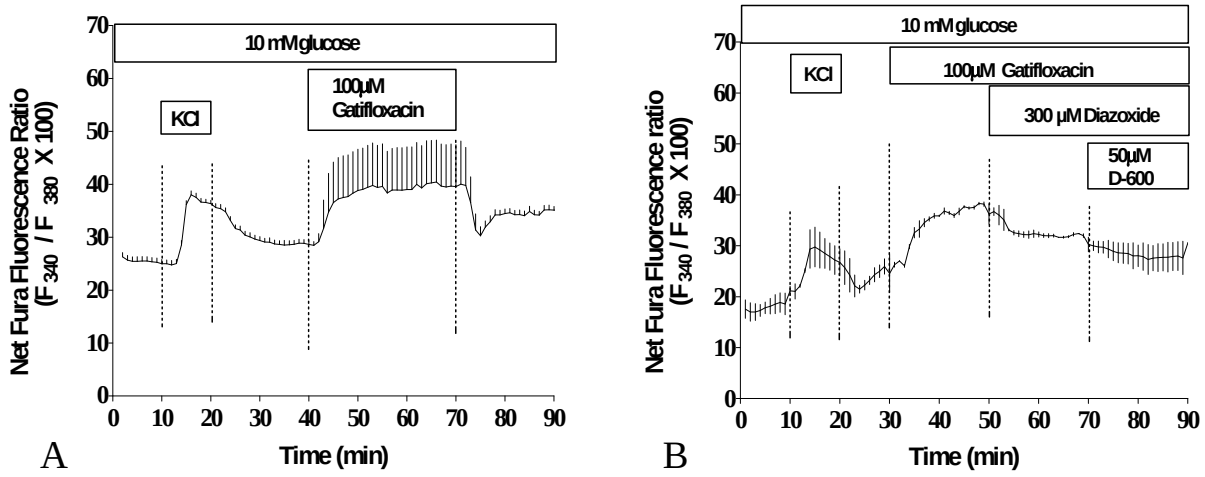


Figure 4.3 Effect of gatifloxacin on $[Ca^{2+}]_i$ in the presence of 10 mM glucose in NMRI mouse islets. A) The functional integrity of the islets was tested from minute 10- 20 by the characteristic response to a maximum K^+-depolarizing stimulus. Perifusion with 100 uM gatifloxacin was performed from minute 30-90. **B)** 300 µM diazoxide (K_{ATP} channel opener) was added from minute 50-70.Additionally, 50 µM of D-600.The $[Ca^{2+}]_i$ was increased to level even exceeding K^+ depolarization. However, it is decreased by diazoxide, even further reduction was observed by addition of D-600, although, in both, $[Ca^{2+}]_i$ did not return to initial values. Data represent means ± SEM based on the analysis of 3 pancreatic islets, for each.

The exposure of cultured islets to 100 µM moxifloxacin in the presence of glucose (5 mM) resulted in an increase in the net Fura fluorescence ratio by 17 units after 2 minutes. The ratio was further increased by 20 units when moxifloxacin concentration was increased to 500 µM. Washout of moxifloxacin led to a clear decrease of the fluorescence ratio to the level prior to exposure to 500 µM. The use of D-600 from minute 70 failed to further reduce the ratio (Fig 4.4). Testing the effect of 100 µM moxifloxacin in the presence of 10 mM glucose showed an increased Ca^{2+} concentration compared with the plateau phase of K^+-induced depolarization. Regarding the kinetics of $[Ca^{2+}]_i$, the ratio was increased after a latency of one minute and reached to a steady state within two minutes (Fig 4.5). In a direct comparison with gatifloxacin (Fig 4.6), moxifloxacin was rather less efficient (increase by about 20 instead of about 27 ratio units); again the effect was of a remarkable fast onset.

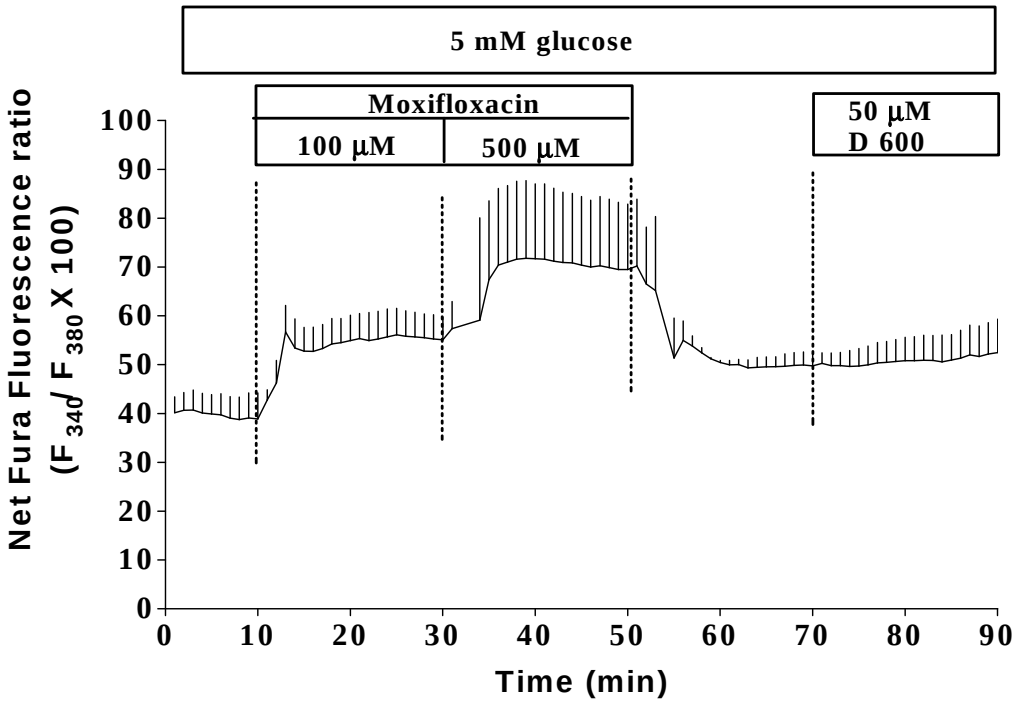


Figure 4.4 Effect of moxifloxacin on $[Ca^{2+}]_i$ in the presence of a basal glucose concentration in NMRI mouse β cells. Cultured normal mouse β cells were perifused with Krebs-Ringer medium containing 5 mM glucose. From minute 10-30, the medium contained 100 µM moxifloxacin and then increased to 500 µM from minute 30-50. After wash-out the basal value of the net Fura fluorescence was established by 50 µM D600. Values are means ± S.E.M of 24 loaded and 29 sham-loaded β cells from 4 experiments each.

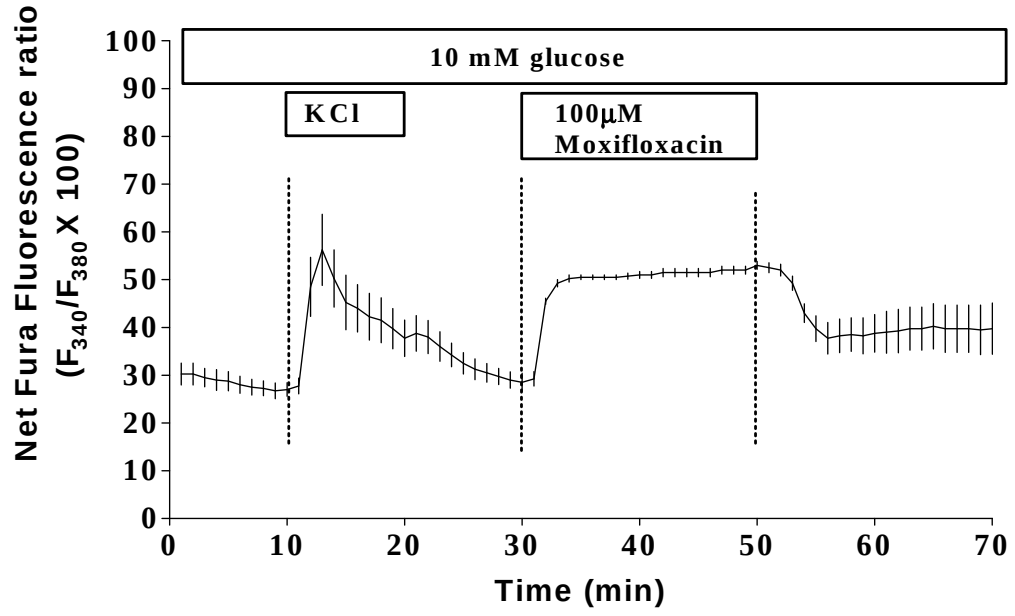


Figure 4.5 Effect of moxifloxacin on $[Ca^{2+}]_i$ in the presence of 10 mM glucose in NMRI mouse islets. The functional integrity of the islets was tested from minute 10-20 by the characteristic response to a maximum K^+-depolarizing stimulus. Perifusion with 100 uM moxifloxacin was performed from minute 30-50 and then washout for 20 minutes. Data represent means ± SEM based on the analysis of 3 pancreatic islets, for each.

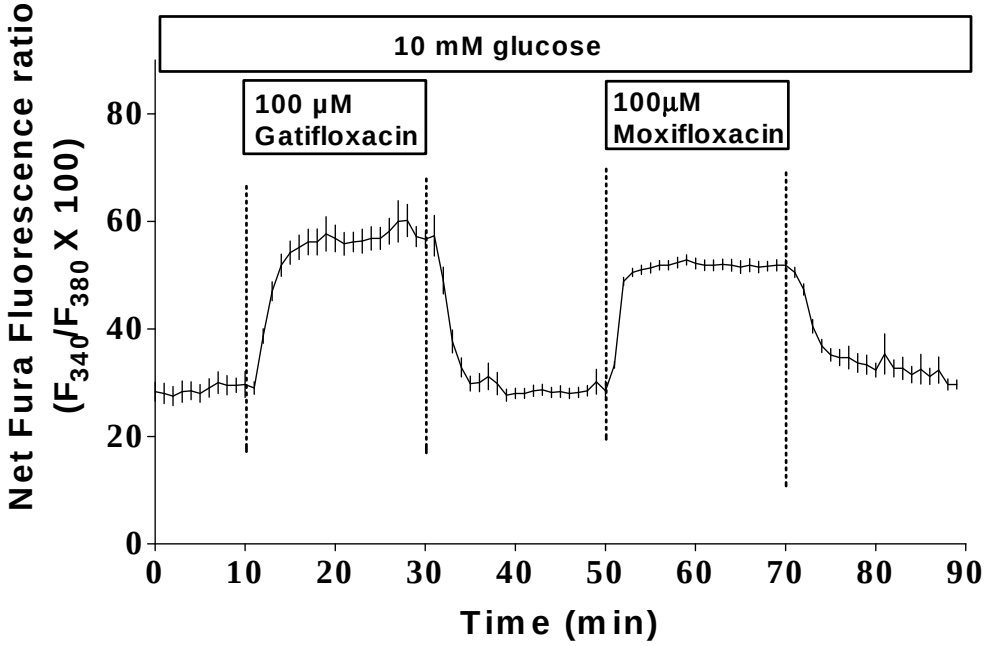


Figure 4.6 Comparison between the effect of gatifloxacin and moxifloxacin on $[Ca^{2+}]_i$ in NMRI mouse islets. Both gatifloxacin and moxifloxacin were used at a concentration of 100 uM in the presence of Krebs Ringer medium containing 10 mM glucose. Gatifloxacin 100 uM was added from minute 10-30, the washed out for 20 minutes before the islets perifused with moxifloxacin 100 uM. Data represent means ± SEM based on the analysis of 3 pancreatic islets, for each.

In the presence of basal glucose concentration, 100 µM ciprofloxacin was not capable of increasing the fluorescence ratio. When the concentration was increased to 500 µM the ratio increased by 20 units within 5 minutes. The washout period showed a partial reversibility with a delayed onset. Then, 50 µM D-600 added from minute 60 produced no further decrease (Fig 4.7). The presence of D-600 in the perifusion medium from the beginning reduced the increment caused by 500 µM ciprofloxacin, but did not completely prevent it as can be seen from the effect of the washout (Fig 4.8). For this reason, the protocol was repeated omitting Ca^{2+} from the perifusion medium. Essentially the same pattern was observed (Fig 4.9A). For comparative purpose, 500 µM tolbutamide (a sulfonylurea drug) was used (Fig 4.9B). It increased the ratio values by nearly 20 units under normal conditions, but was totally ineffective in the absence of Ca^{2+} in the perifusion medium.

Next, the effect of 100 and 500 µM ciprofloxacin in the presence of 10 mM glucose was examined. After a lag time of about 15 minutes, a moderate increase by 5 units became visible. The values were further increased by 20 units when the concentration was increased to 500 µM. Washout (from minute 50 on) promptly but incompletely reduced the Fura ratio. The addition of D-600 from minute 70-90 (Fig 4.10) was not able to further reduce the ratio.

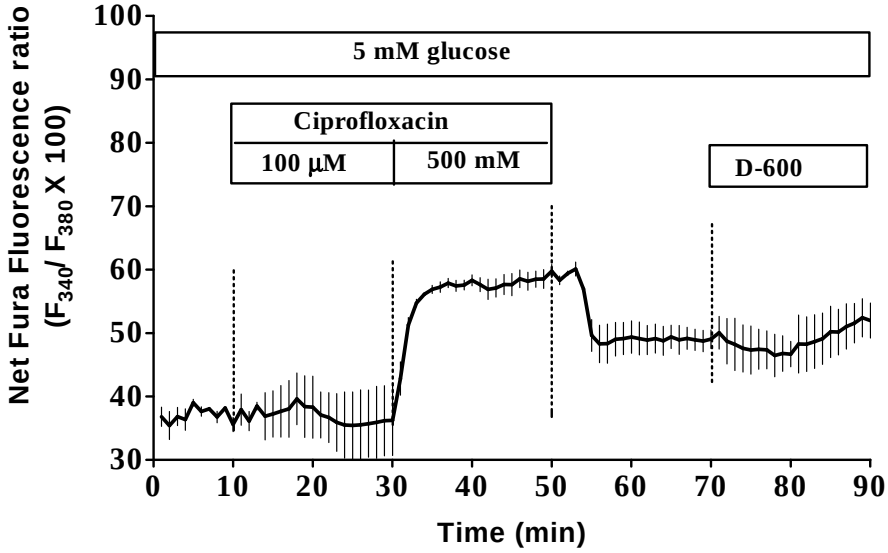


Figure 4.7 Effect of ciprofloxacin on $[Ca^{2+}]_i$ in the presence of a basal glucose concentration in NMRI mouse islets. Cultured normal mouse islets were perifused with Krebs-Ringer medium containing 5 mM glucose. Ciprofloxacin conc. was changed from 100 µM (min. 10-30) to 500 µM from min. 30 to 50. The level of $[Ca^{2+}]_i$ is still unexpectedly high even after wash-out continued for 30 minutes. Values represent means ± SEM based on the analysis of 3 pancreatic islets, for each.

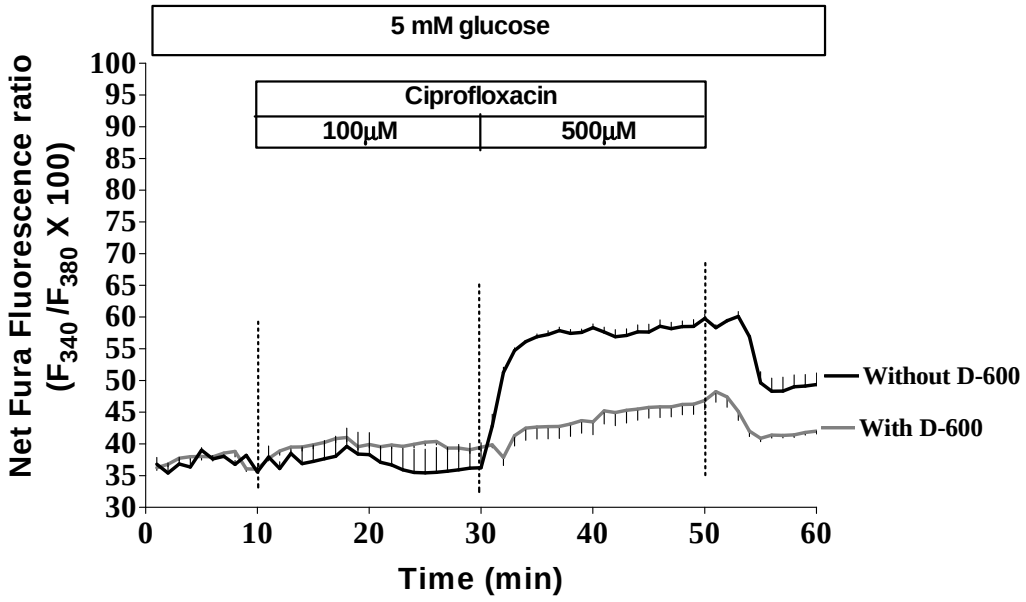


Figure 4.8 Effect of two concentrations of ciprofloxacin on $[Ca^{2+}]_i$ in the presence of a basal glucose concentration in the presence and absence of D-600 in NMRI mouse islets. Cultured normal mouse islets or β cells were perifused with Krebs-Ringer medium containing 5 mM glucose. Ciprofloxacin conc. was changed from 100 µM (min. 10-30) 500 µM from min. 30 to 50. The level of $[Ca^{2+}]_i$ is reduced by the addition of D-600, however not return to the level prior to ciprofloxacin addition to the perifusion medium. Values represent means ± SEM based on the analysis of 3 pancreatic islets, for each.

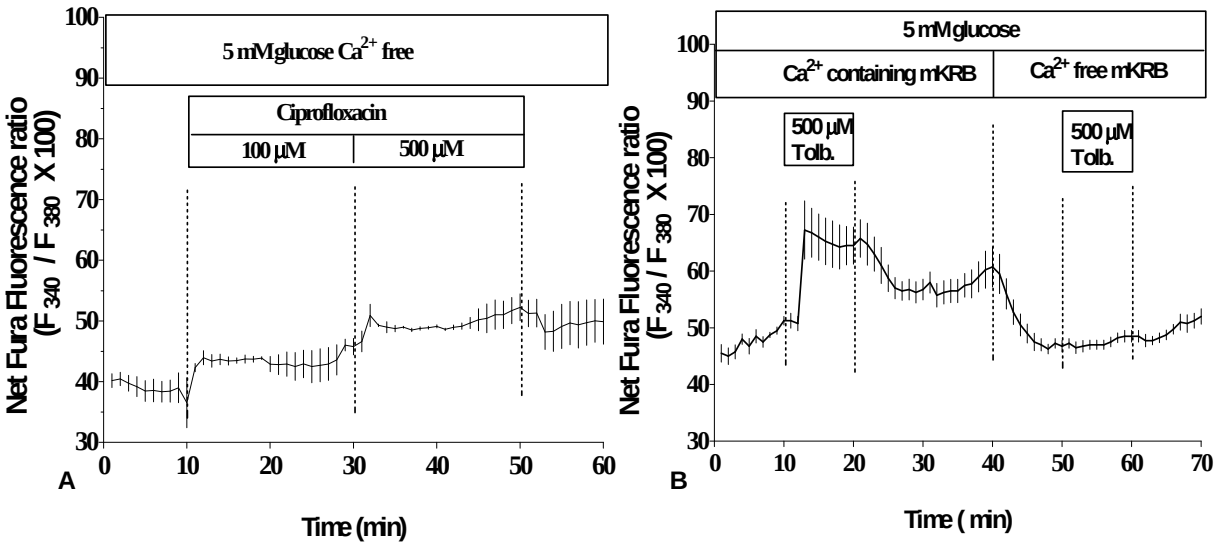


Figure 4.9 Effect of two concentrations of ciprofloxacin on $[Ca^{2+}]_i$ in the presence of a basal glucose concentration in the presence and absence of Ca^{2+} in NMRI mouse islets. **A)** The effect of ciprofloxacin on the $[Ca^{2+}]_i$ in the presence of a basal glucose concentration in a calcium free Krebs-Ringer medium compared to the effect of tolbutamide 500 µM. Ciprofloxacin conc. was changed from 100 µM (min. 10-30) to 500 µM from min. 30-50. The level of $[Ca^{2+}]_i$ is not markedly different by absence of calcium from the medium. **B)** The effect of tolbutamide 500 µM was completely abolished when the islets perifused with calcium free medium. Values represent means ± SEM based on the analysis of 3 pancreatic islets, for each.

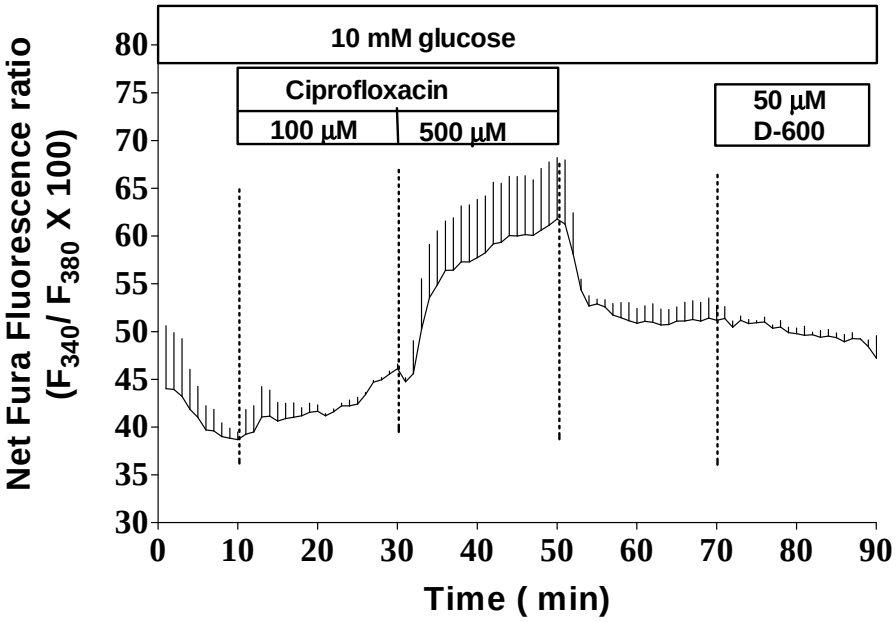


Figure 4.10 Effect of two concentrations of ciprofloxacin on $[Ca^{2+}]_i$ in the presence of 10 mM glucose in NMRI mouse islets. Effect of ciprofloxacin on the $[Ca^{2+}]_i$ in the presence of 10 mM glucose. Cultured normal β cells were perifused with Krebs-Ringer medium containing 10 mM glucose. Ciprofloxacin conc. was changed from 100 µM (min. 10-30) 500 µM from min. 30 to 50. $[Ca^{2+}]_i$ is not so much increased using 100 µM while only increased markedly at 500 µM. D-600 was ineffective to reduce the net ratio values considerably. Values are means ± S.E.M of 24 loaded and 20 sham-loaded β cells from 4 experiments each.

4.1.2 K_{ATP} channel-independent effect of fluoroquinolones on the $[Ca^{2+}]_i$

The question whether the K_{ATP} channel blockade is indispensible for the $[Ca^{2+}]_i$ increase was investigated by measuring the effect of gatifloxacin, moxifloxacin, and ciprofloxacin on SUR1 KO islets. The microfluorometric measurements of $[Ca^{2+}]_i$ from K_{ATP} channel-deficient SUR1 KO islets were carried out under the same experimental conditions as used for the normal NMRI islets. The net Fura fluorescence ratio was calculated as outlined before. Compared to islets from NMRI mice, the fluorescence ratio showed higher ratio values before the addition of the test compounds. In all experiments, the islets were perifused with mKRB containing 10 mM glucose.

The SUR1 KO islets showed a modest (non typical) response to a high K^+ concentration. The ratio was increased only from 53 to 64 units. In contrast, 100 µM gatifloxacin induced a marked increase of the Fura-fluorescence ratio (minute 30-50). After a transient reduction, it increased progressively and reached a steady state at the end of 20 minutes perfusion. 50 µM D-600 seemed to be ineffective in reducing the ratio. When gatifloxacin was withdrawn from the medium (minute 60), a prompt and massive reduction occurred (Fig 4.11).

The perifusion with 100 µM moxifloxacin (from minute 30-50) in the presence of 10 mM glucose resulted in an instant increase in the net Fura fluorescence ratio from 58 to 72 units. The use of D-600 from minute 50-60 failed to significantly reduce the ratio. However from minute 60-70, the washout in the presence of D-600 decreased the value to 63 units; though not to pre-exposure level. As in the preceding experiments with SUR1 KO islets, a strong KCl depolarization from minute 10-20 showed only a moderate increase in the fluorescence ratio by about10 units (Fig 4.12).

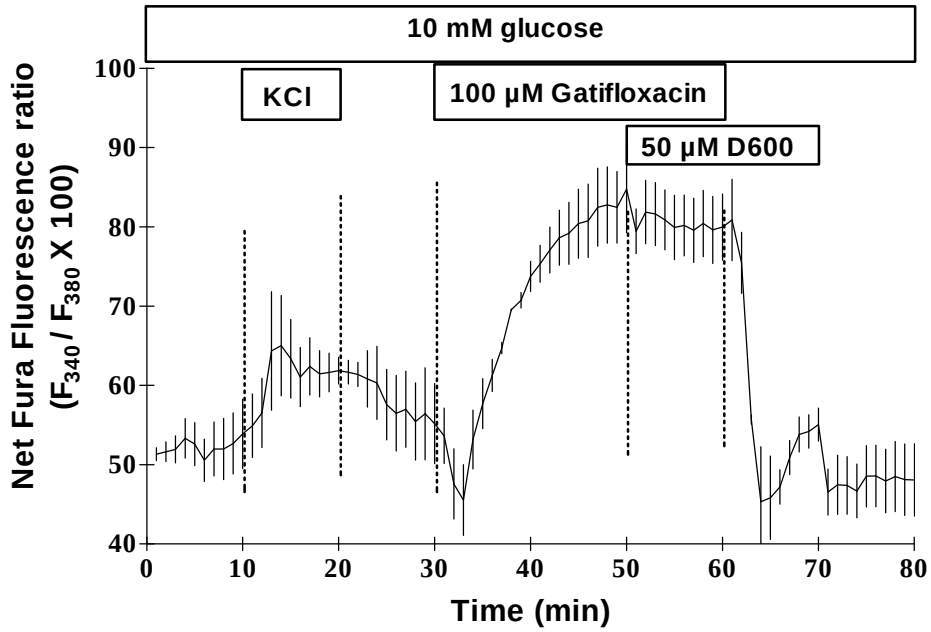


Figure 4.11 Effect of gatifloxacin on the $[Ca^{2+}]_i$ in the presence of 10 mM glucose in SUR1KO islets. The functional integrity of the islets was tested from minute 10- 20 by the characteristic response to a maximum K^+-depolarizing. Gatifloxacin 100 µM added from minute 30 to 60 interrupted by 50 µM of D-600 from minute 50-60. Values are means ± S.E.M from a total of 3 pancreatic islets Fura loaded and 3 sham loaded islets.

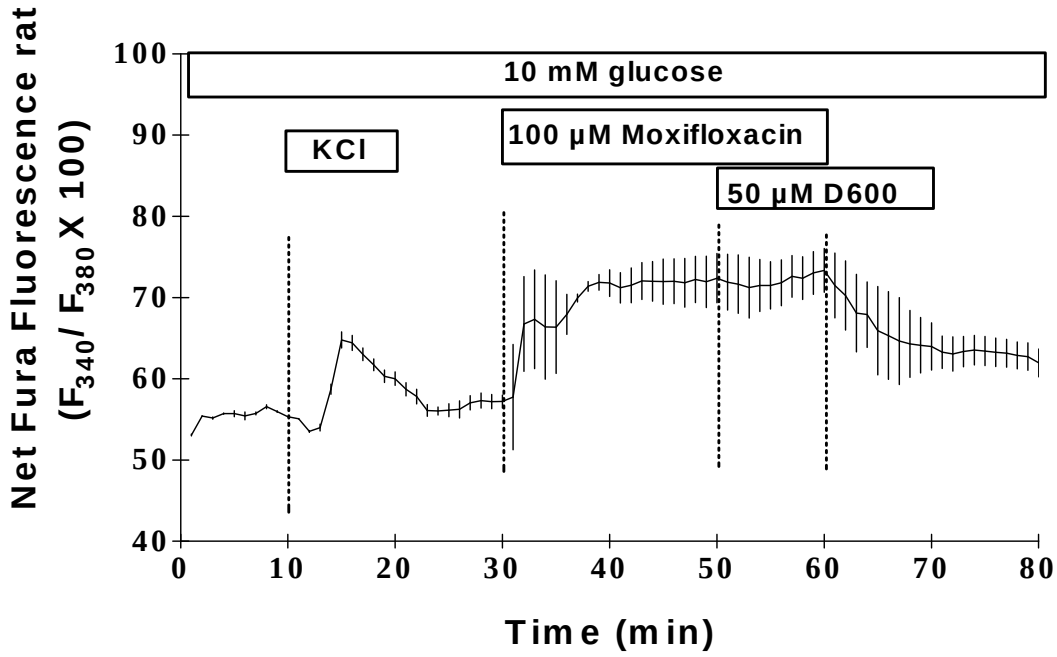


Figure 4.12 Effect of moxifloxacin on the $[Ca^{2+}]_i$ in the presence of 10 mM glucose in SUR1KO islets. The functional integrity of the islets was tested from minute 10- 20 by the characteristic response to a maximum K^+-depolarizing. Perfusion with moxifloxacin 100 µM from minute 30-60 interrupted by 50 µM of D-600 from minute 50-60. Values are means ± S.E.M from a total of 3 pancreatic islets Fura loaded and 3 sham loaded islets.

Perifusion with 100 µM ciprofloxacin from minute 30-50 induced a moderate increase in the net Fura-fluorescence ratio from 60 to 81 units with a rapid initial and a protracted second response phase. The effect of D-600 from minute 50-60 minutes resulted in a marginal reduction of 5 units. The washout in the presence of D-600 resulted in a level that was close to pre-stimulatory conditions (minute 60-70). After completion of perifusion with D-600, the course of fluorescence ratio did not change (Fig 4.13).

The magnitude of this increase in $[Ca^{2+}]_i$ caused by fluoroquinolones in SUR1 KO mouse islets was gatifloxacin > moxifloxacin > ciprofloxacin. Reversibility was observed with both gatifloxacin and ciprofloxacin but not with moxifloxacin.

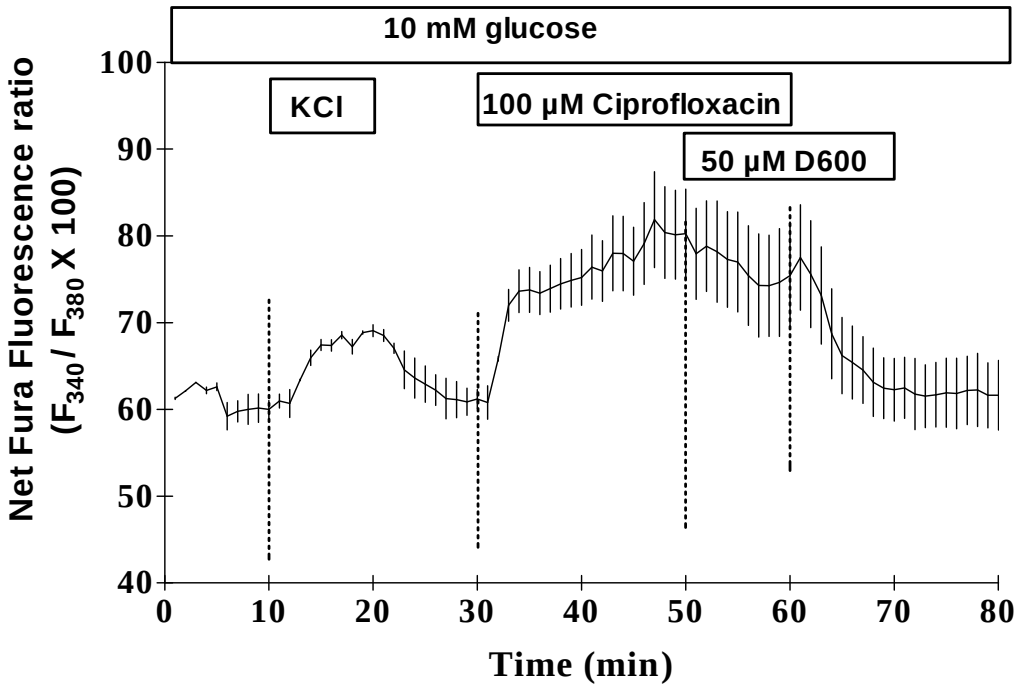


Figure 4.13 Effect of ciprofloxacin on the $[Ca^{2+}]_i$ in the presence of 10 mM glucose in SUR1 KO islets. The functional integrity of the islets was tested from minute 10- 20 by the characteristic response to a maximum K^+-depolarizing. Perfusion with ciprofloxacin 100 µM from minute 30-60 interrupted by 50 µM of D-600 from minute 50-60. Values are means ± S.E.M from a total of 3 pancreatic islets Fura loaded and 3 sham loaded islets.

4.2 Effect of fluoroquinolones on the autofluorescence of reducing equivalents

4.2.1 NAD(P)H autofluorescence measurement in NMRI mouse islets

An increase in glucose concentration to 20 mM resulted in an increase in signal intensity by 35 ± 5 % within 300 s and subsequently returned to the prestimulatory after a latency of 100 s after glucose had been removed from the medium. For more accurate comparison of the response to 20 mM glucose, the islets were also perifused with mKRB without glucose for 60 minutes where the intensity continued to decline to reach 90 % at 2400 s and 85 % at the end of perifusion. The raw data for NAD(P)H fluorescence (in arbitrary units) was presented (Fig 4.14A). However, variability in the size of pancreatic islets as well as the size of the illuminated area made the intensities quite variable, and so the resulting scatters of the mean values rendered a demonstration of significant drug effects difficult. For these reasons, the values were normalized to 100 % with respect to the last prestimulatory value i.e. before the change of glucose concentration to 20 mM. The increase at the end of perifusion with 20 mM glucose in NMRI islets was 45 % relative to the control islets (Fig 4.14B).

To confirm that the signal of NAD(P) H fluorescence is mitochondrial in origin, CCCP was used which blocked the electron transport chain leading to maximal reduction of all components of the chain, including NAD(P)H. For this reason, 5 µM CCCP was added in presence of 20 mM glucose, where it reduced gradually the fluorescence to the level prior to exposure to CCCP by the end of 20 min perifusion (Fig 4.15).

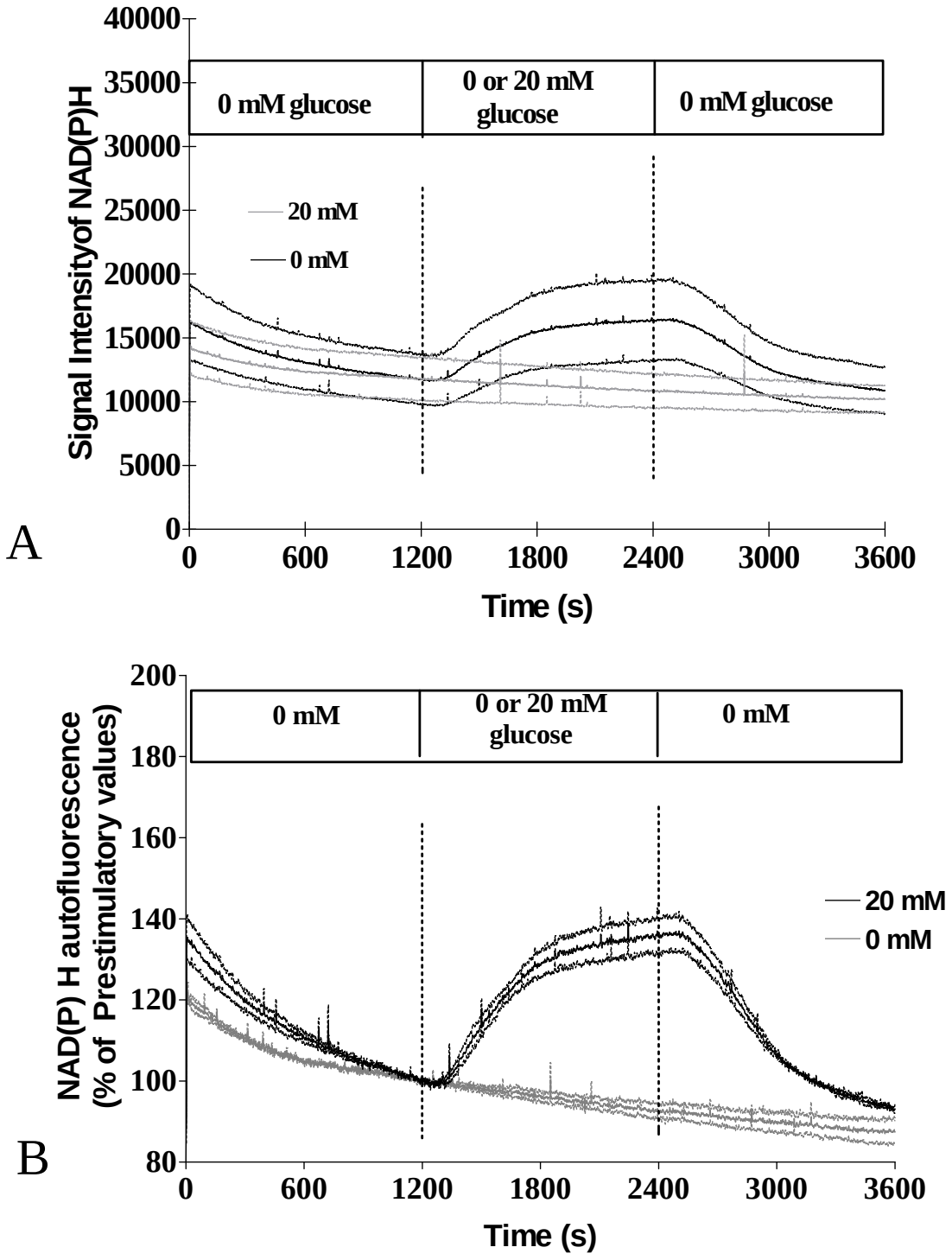


Figure 4.14 Changes in NAD(P)H signal in response to 20 mM glucose in NMRI mouse islets at 36 °C. A) Increase in NAD(P)H fluorescence intensity in pancreatic islets of the NMRI mouse by glucose at 36 ° C. The islets were first perifused with Krebs-Ringer medium, containing the 0 mM glucose for 20 minutes (second 0 to 1200). This was followed by an increase in the glucose concentration to 20 mM (second from 1200 to 2400) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The signal intensity (arbitrary units) is plotted against time in seconds. The grey line shows the effect of perifusion of the islets with Krebs-Ringer solution 0 mM glucose. Values are means ± SEM of six experiments for each. Pancreatic islets were obtained from 2 different preparations. **B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

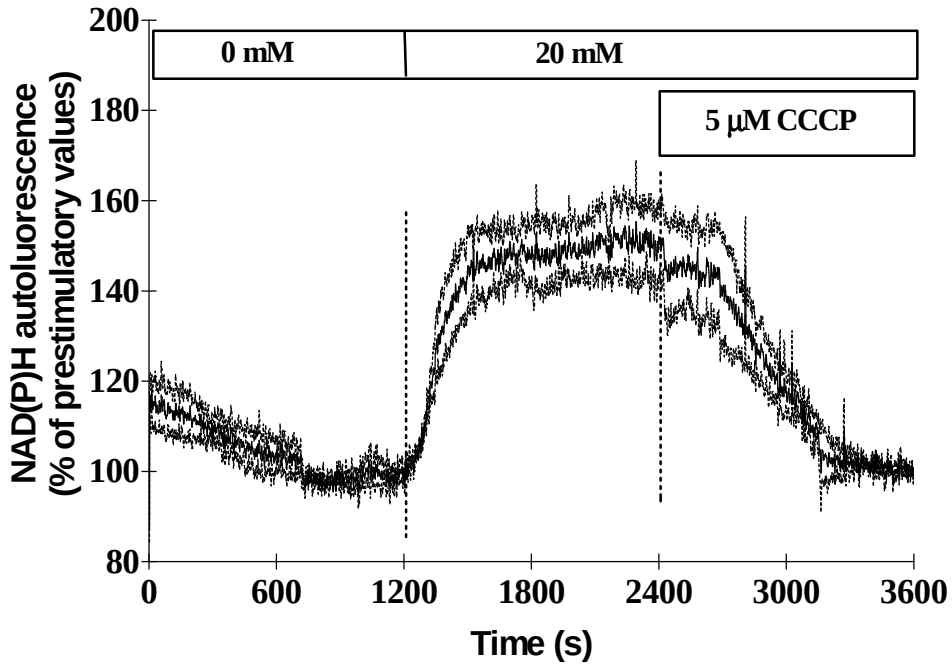


Figure 4.15 Changes in NAD(P)H signal in response to 20 mM glucose with CCCP in NMRI mouse islets at 36 °C. Effect of CCCP on the increased NAD(P)H fluorescence intensity brought about by 20 mM glucose in pancreatic islets of the NMRI mouse. The islets were first perifused with Krebs-Ringer medium, containing the 0 mM glucose for 20 minutes (second 0 to 1200). This was followed by an increase in the glucose concentration to 20 mM (second from 1200 to 2400) and from 2400 seconds CCCP 5 µM for the last 20 minutes of perifusion. Values are normalized by setting the last pre-stimulatory value (20 min) to 100%. Values are means ± SEM of 3 experiments.

The following set of experiments, was performed to assess the influence of gatifloxacin, moxifloxacin, and ciprofloxacin (100 µM each) on NAD(P)H autofluorescence in response to glucose stimulation. The autofluorescence of each compound was measured both in the presence of 20 mM glucose and of 0 mM glucose.

The addition of gatifloxacin (100 µM) in the presence of 20 mM glucose increased the signal intensity 4 times after a latency of 120 s before reaching a steady state at 4800 second. When 0 mM glucose was used from the start of perifusion, gatifloxacin still elevated the intensity with the effect size and lag time similar to that seen when 20 mM glucose was used (Fig 4.16A). When the glucose concentration was increased to 20 mM the signal intensity of NAD(P)H increased to 45 % of the pre-stimulatory values (Fig 4.16B).

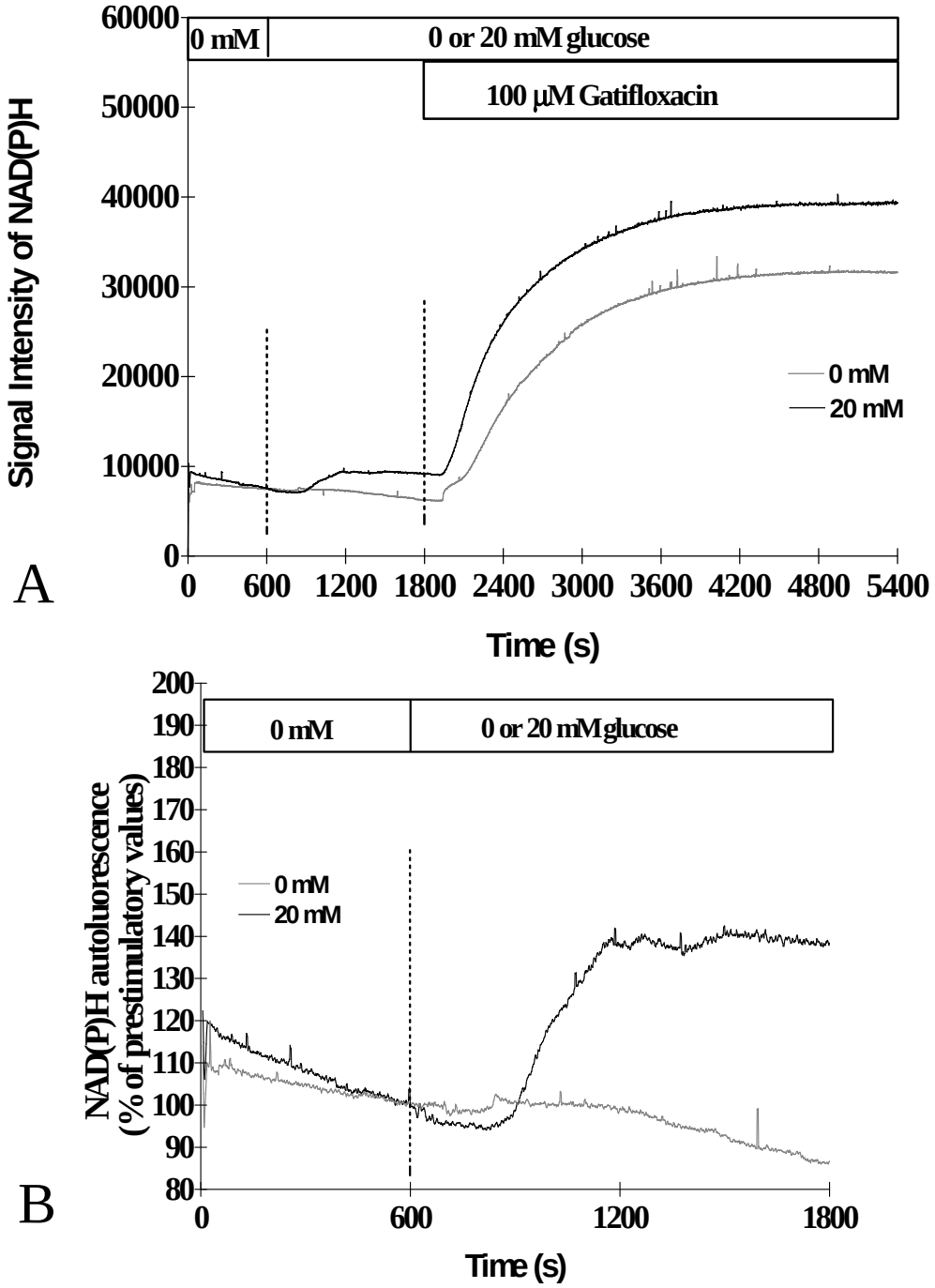


Figure 4.16 Changes in NAD(P)H signal in response to gatifloxacin in the presence of 20 mM glucose in NMRI mouse islets at 36 °C. A) The islets were perifused with KR-medium contained 0 mM glucose (Second 0-600). In the second 1200 (minute 10-30), glucose concentration increased to 20 mM. Then, 100 µM gatifloxacin was added from minute 30 for one hour (5400 second). **B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (10 min) to 100%. The figure shows the islets response to increase glucose concentration prior to application of gatifloxacin. The islets were obtained from 2 different preparations. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose then, gatifloxacin was added from minute 30 till the end of perifusion.

The same protocol was applied to moxifloxacin where it increased the signal intensity about 20 times after a latency of 190 s (slower kinetics than gatifloxacin). A steady state had been reached only before termination of perifusion by 20 minutes. When 0 mM glucose was used from the beginning of perifusion, still moxifloxacin elevated the intensity 25 times with a lag time of 80 s faster than that seen when 20 mM glucose was used (Fig 4.17A). Increasing glucose concentration resulted in increase of the signal intensity of NAD(P)H by 50% of the prestimulatory values (Fig 4.17B).

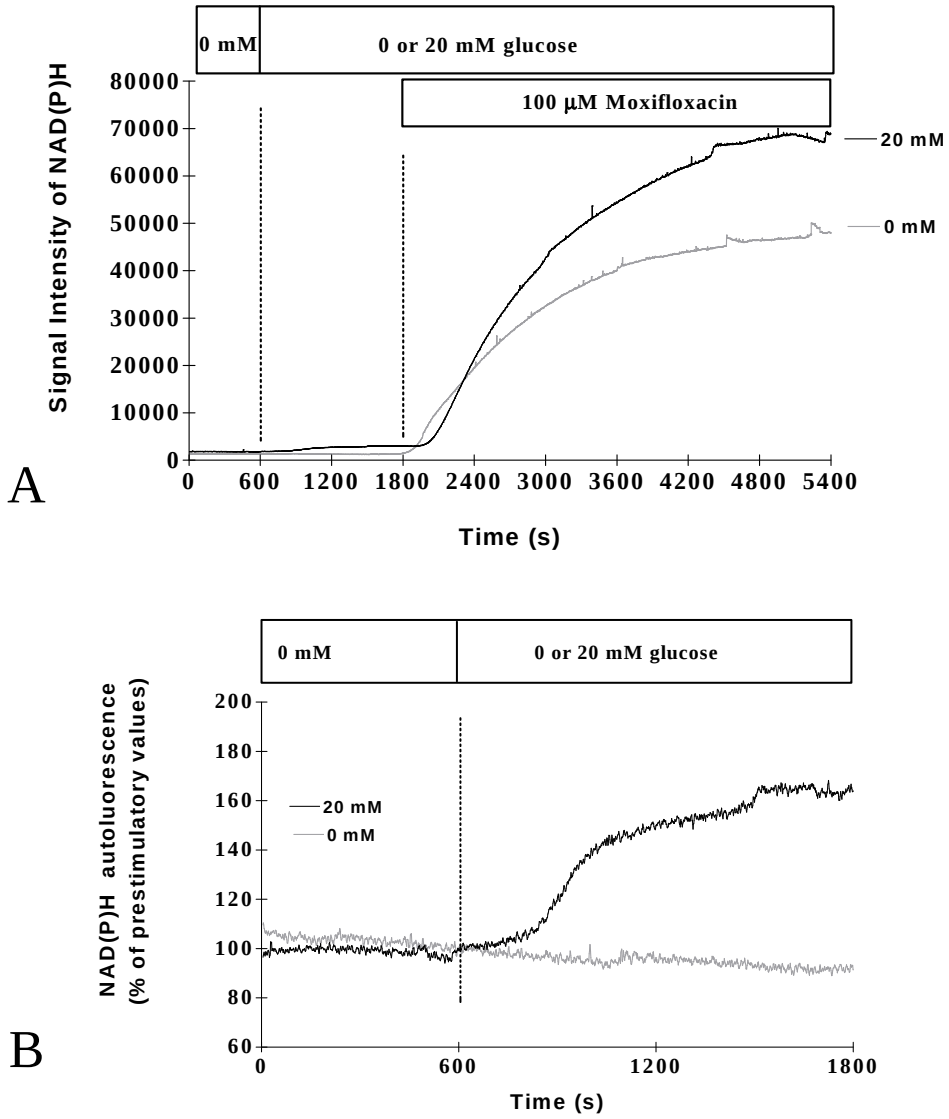


Fig 4.17 Changes in NAD(P)H signal in response to moxifloxacin in the presence of 20 mM glucose in NMRI mouse islets at 36 °C. A) The islets were perifused with KR-medium contained 0 mM glucose (Second 0-600). In the second 1200 (minute 10-30), glucose concentration increased to 20 mM. Then, 100 µM moxifloxacin was added from minute 30 for one hour (5400 second). **(B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (10 min) to 100%. The figure shows the islets response to increase glucose concentration prior to application of moxifloxacin. The islets were obtained from two different preparations. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose then, moxifloxacin was added from minute 30 till the end of perifusion.

When ciprofloxacin was added in presence of 20 mM glucose for one hour, the fluorescence was doubled after 150 s without reaching a steady state till the end of perifusion. Using 0 mM glucose, ciprofloxacin also doubled the signal intensity (Fig 4.18A). The perifusion with 20 mM glucose after 10 minutes increased NAD(P)H autofluorescence by 30-45% (Fig 4.18B).

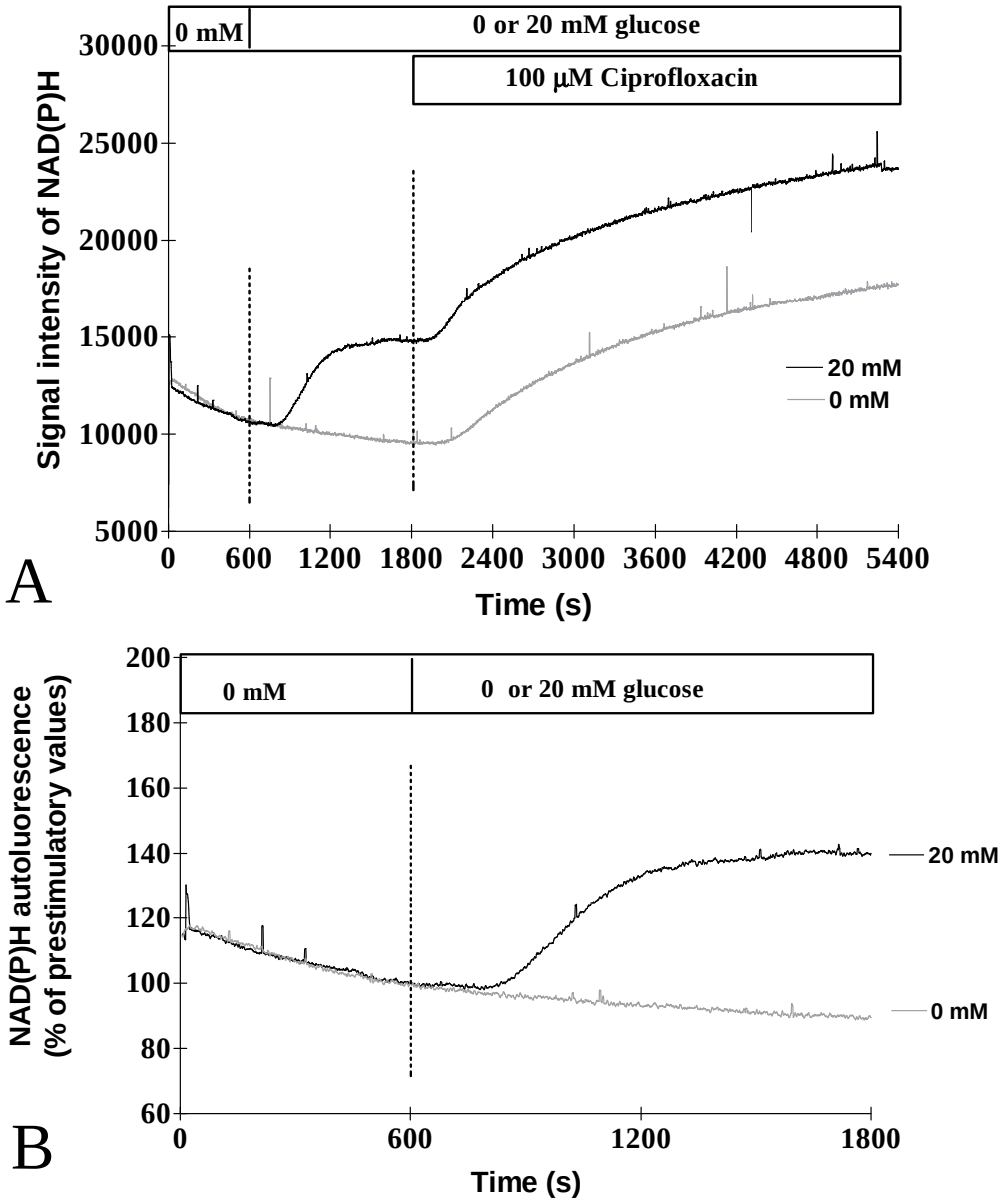


Figure 4.18 Changes in NAD(P)H signal in response to ciprofloxacin in the presence of 20 mM glucose in NMRI mouse islets at 36 °C. A) The islets were perifused with KR-medium contained 0 mM glucose (Second 0-600). In the second 1200 (minute 10-30), glucose concentration increased to 20 mM. Then, 100 µM ciprofloxacin was added from minute 30 for one hour (5400 second). **(B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (10 min) to 100%. The figure shows the islets response to increase glucose concentration prior to application of ciprofloxacin. The islets were obtained from two different preparations. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose then, ciprofloxacin was added from minute 30 till the end of perifusion.

4.2.2 Comparison between fluoroquinolones regarding NAD (P) H autofluorescence

The pattern of NADH increase caused by each drug was quite different. When the values of NAD(P)H signal intensity were normalized at 1800 seconds ,the extents of increase caused by ciprofloxacin, gatifloxacin and moxifloxacin were 200 %, 125 %, and 138 % respectively. Ciprofloxacin had the least initial fluorescence intensity, gradually increasing without reaching a steady state. Whereas moxifloxacin initially showed extremely high values with very slow kinetic profile only assuming a steady state after 40 minutes of perifusion. Gatifloxacin kinetic profile started like ciprofloxacin concerning the initial fluorescence but differently reaching a steady state. To distinguish whether the largely different signal intensities were due a different accumulation of the compounds in the islet cells or to different fluorescence intensity in the NAD(P)H emission range, the fluorescence intensities of the 3 compounds was measured in the absence of islets but otherwise exactly the same experimental conditions (Fig 20). At an equimolar concentration moxifloxacin had a 25 fold and gatifloxacin 5.4 fold higher fluorescence intensity compared to ciprofloxacin. Corrected for these values the rank order of accumulation was cipro > gati > moxi. However, moxifloxacin showed the most marked increase of accumulation in the presence of 20 mM glucose (40.2% vs. 17.6% for both gati- and ciprofloxacin). The time required for NAD(P)H to reach to half its peak value for each drug was compared. While the half-maximal increase of fluorescence during the chamber perifusion was practically the same for all compounds (240 s), the accumulation velocity by islets differed significantly (for gatifloxacin ~ 500 s, ciprofloxacin ~ 900 s, and moxifloxacin ~ 1100s). The time required for NAD(P)H to return to half its peak value (50% recovery time) was also analyzed as shown in Table 4.1.

Dividing the net fluorescence by the relative compound autofluorescence yields the normalized autofluorescence which is a measure of the amount of fluoroquinolone accumulated by the islet cells. The amount of ciprofloxacin in pancreatic islets is 3.7 fold higher and that of gatifloxacin is 2.1 fold higher than that of moxifloxacin.

Table 4.1 Different accumulation of fluoroquinolones in pancreatic islets

	Net fluorescence signal at 0 mM glucose	Net fluorescence signal at 20 mM glucose	Glucose-dependent increase	Relative compound fluorescence	Normalized fluorescence at 20 mM glucose	Relative islet concen-tration
Gatifloxacin	24.000	29.500	+22.9 %	5.4	5504	2.1
Ciprofloxacin	8.500	9.500	+11.8 %	1.0	9500	3.7
Moxifloxacin	46.500	64.500	+38.7 %	25.0	2580	1.0

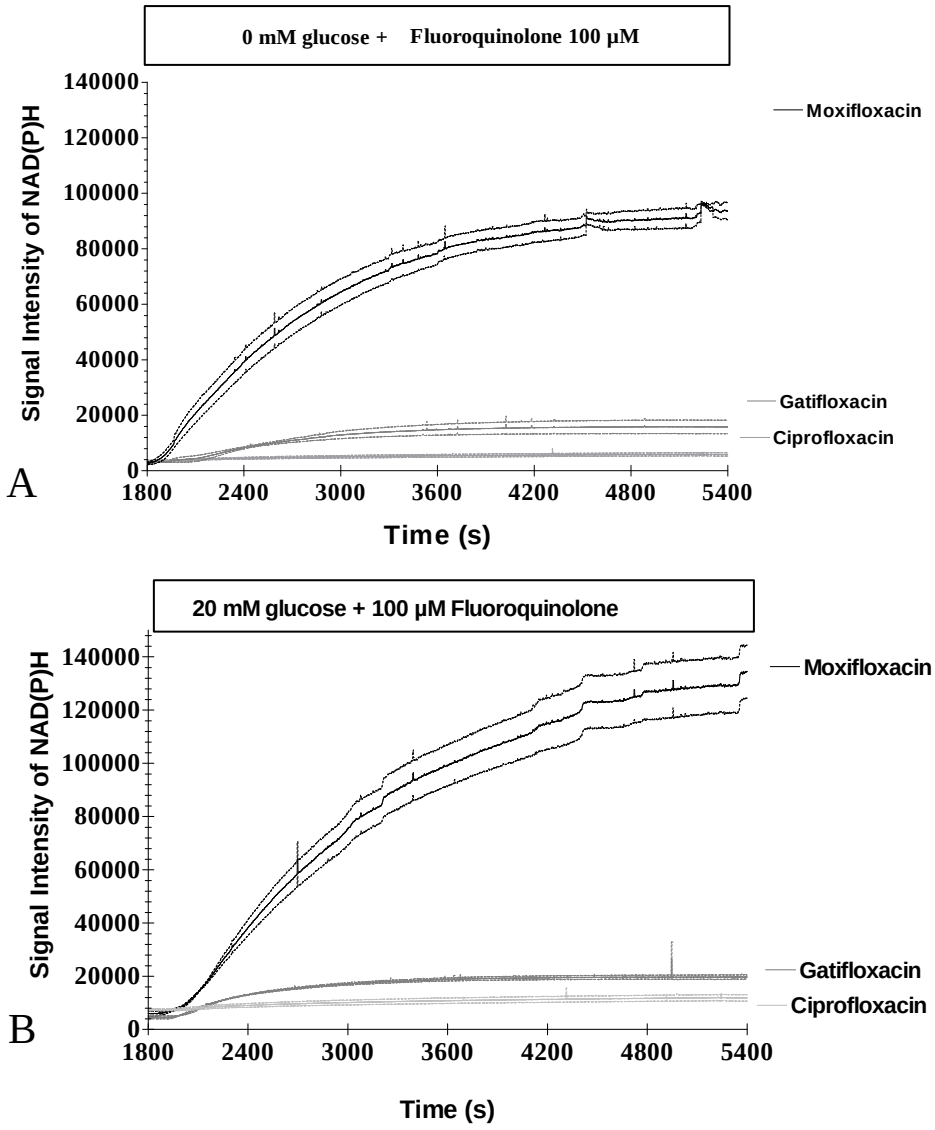


Figure 4.19 and Comparison between the 3 compounds in presence of 0 mM (A) and 20 mM glucose (B). It seems that the enhanced energetic caused by increasing glucose concentration affect the endogenous fluorescence of the fluoroquinolones.

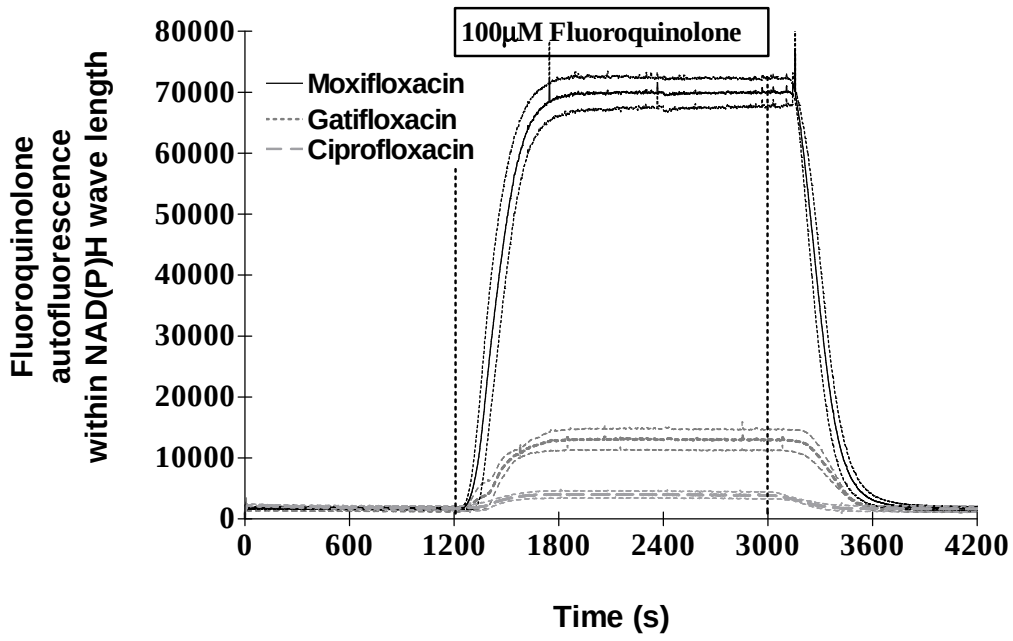


Figure 4.20 The autofluorescence of Fluoroquinolones 100 µM in the presence of 20 mM glucose. The perifusion chamber contained no pancreatic islets.

4.2.3 Effects on NAD(P)H autofluorescence in pancreatic islets pre-incubated with fluoroquinolones

4.2.3.1 NMRI mice

Pre-incubation protocol was performed as the used fluoroquinolones had their own intrinsic fluorescence that interfered with the direct measurements of NAD(P)H signal intensity and to avoid the interference of the glucose metabolism with the cellular accumulation of the fluoroquinolones. In this type of experiments, the pancreatic islets were pre-incubated for one hour with 100 µM of each of the three drugs and then the islets were exposed to a challenge with 20 mM glucose followed by a 20 min perfusion at 0 mM glucose to test for reversibility. Similarly, washout experiments were performed by continuous perifusion with 0 mM glucose.

When NMRI islets were pre-incubated with gatifloxacin, the autofluorescence was increased by 15 ± 10 % of the pre-stimulatory values and 30 % relative to the washout experiment. The autofluorescence values returned after glucose withdrawal. When perifusion was continued with 0 mM glucose, the values were progressively decreased to reach 72 % of the pre-stimulatory values (Fig 4.21).

When NMRI islets were pre-incubated with moxifloxacin, the autofluorescence of NAD(P)H began to decrease so fast. Increasing glucose concentration to 20 mM

seemed to be ineffective to change NAD(P)H values throughout the whole 20 minutes of perifusion. The kinetic profile in the remainder 20 minutes of perifusion was parallel to the washout experiment (Fig 4.22).

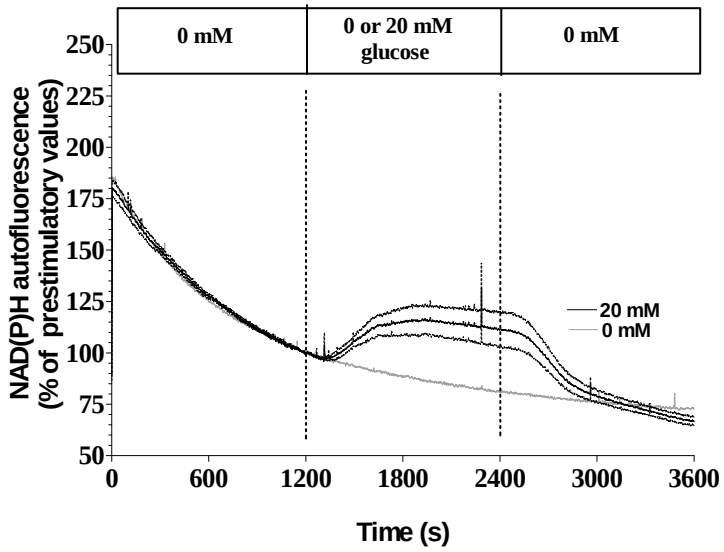


Figure 4.21 Changes in NAD(P)H signal in response to 20 mM glucose in NMRI mouse islets pre-incubated with 100 µM gatifloxacin at 36 °C. The islets were perifused with Krebs-Ringer medium containing 0 mM glucose for 20 minutes. The increase in NAD(P)H fluorescence was caused by glucose concentration 20 mM from second 1200 for 20 minutes. The effect is compared with wash out of gatifloxacin i.e. exposure of the islets to mKRB containing 0 mM during the whole 60 minutes protocol (the grey line) .Values are means ± SEM of 3 experiments for each. The values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

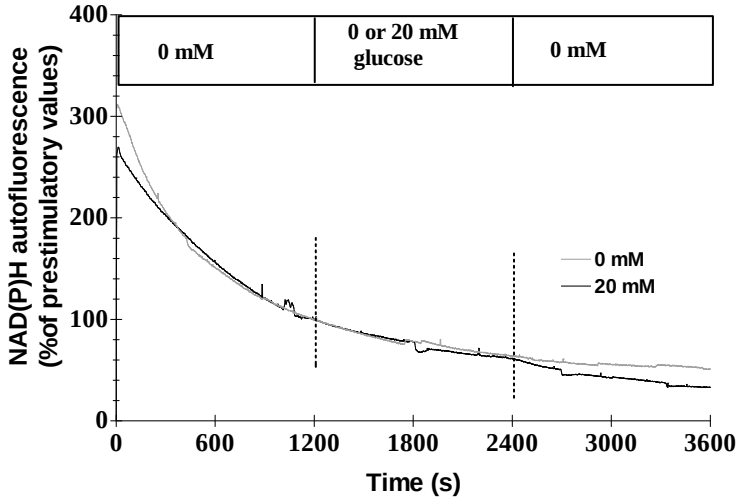


Figure 4.22 Changes in NAD(P)H signal in response to 20 mM glucose in NMRI mouse islets pre-incubated with 100 µM moxifloxacin at 36 °C. The islets were perifused with Krebs-Ringer medium containing 0 mM glucose for 20 minutes. The increase in NAD(P)H fluorescence was caused by glucose concentration 20 mM from second 1200 for 20 minutes. The effect is compared with wash out of moxifloxacin i.e. exposure of the islets to mKRB containing 0 mM during the whole 60 minutes protocol (the grey line) .Values are means ± SEM of 6 experiments for each. The values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

When NMRI islets were pre-incubated with ciprofloxacin, NAD(P)H autofluorescence decreased slowly until glucose concentration was increased to 20 mM where the values were increased by 45-60 % of the pre-stimulatory values and 60 % relative to the was out experiment. The values returned to pre-stimulatory level but not the level of the washout experiments (Fig 4.23).

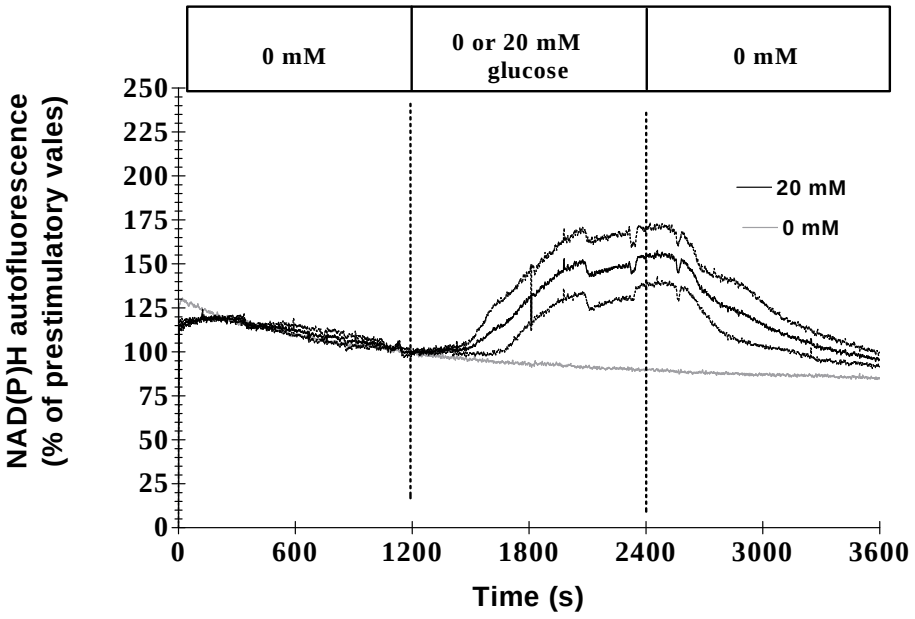


Figure 4.23 Changes in NAD(P)H signal in response to 20 mM glucose in NMRI mouse islets pre-incubated with 100 µM ciprofloxacin at 36 °C. The islets were perifused with Krebs-Ringer medium containing 0 mM glucose for 20 minutes. The increase in NAD(P)H fluorescence was caused by glucose concentration 20 mM from second 1200 for 20 minutes. The effect is compared with wash out of ciprofloxacin i.e. exposure of the islets to mKRB containing 0 mM during the whole 60 minutes protocol (the grey line) .Values are means ± SEM of 3 experiments for each. The values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

4.2.3.2 Islets of SUR1 KO mice

To test whether the inhibitory effect of the fluoroquinolones on the mitochondrial energetics was dependent on their effect on K_{ATP} channels, the NAD(P)H measurements were performed on islets from SUR1KO islets, which lack functional K_{ATP} channels. The cultured SUR1 KO islets were pre-incubated for 1 h in the presence of 100 µM of each of the three drugs. As shown in Fig 4.24 A and B, the islets showed a response not less than that observed with normal NMRI islets. Increasing glucose concentration to 20 mM increased NAD(P)H autofluorescence by 30 % of the pre-stimulatory values and 45 % relative to the washout experiment. Further kinetics revealed the return of values to the pre-stimulatory level.

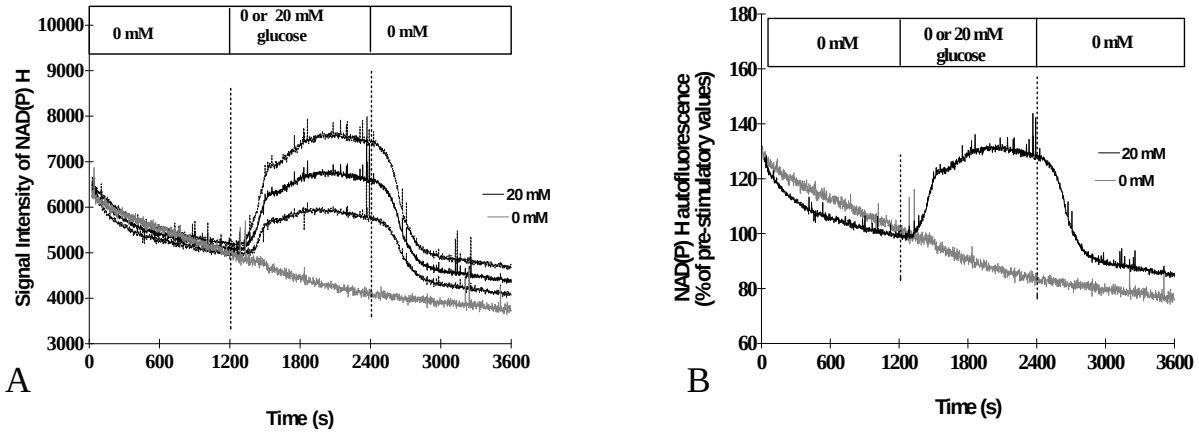


Figure 4.24 Change in NAD(P)H fluorescence intensity in response to 20 mM glucose in SUR1 KO mouse islets at 36 °C. A) The islets were first perifused with Krebs-Ringer medium, containing the 0 mM glucose for 20 minutes (second 0 to 1200). This was followed by an increase in the glucose concentration to 20 mM (second from 1200 to 2400) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The signal intensity (arbitrary units) is plotted against time in seconds. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose. Values are means ± SEM of six experiments for each. The six pancreatic islets were obtained from 2 different preparations. **B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

When the islets of SUR1 KO mice were pre-incubated with gatifloxacin, NAD(P)H autofluorescence decreased rapidly until glucose concentration was increased to 20 mM, where the values increased by 4% of the prestimulatory values while 15 % relative to the washout experiment. The fluorescence values decreased to 90 % in the washout experiment (Fig 4.25 A and B).

When the islets of SUR1 KO mice were pre-incubated with moxifloxacin, the autofluorescence of NAD(P)H started to decrease so fast and again glucose concentration 20 mM failed to increase NAD(P)H values throughout the whole 20 minutes of perifusion. The kinetic profile in the remainder time of perifusion was parallel to the washout experiment (Fig 4.26 A and B).

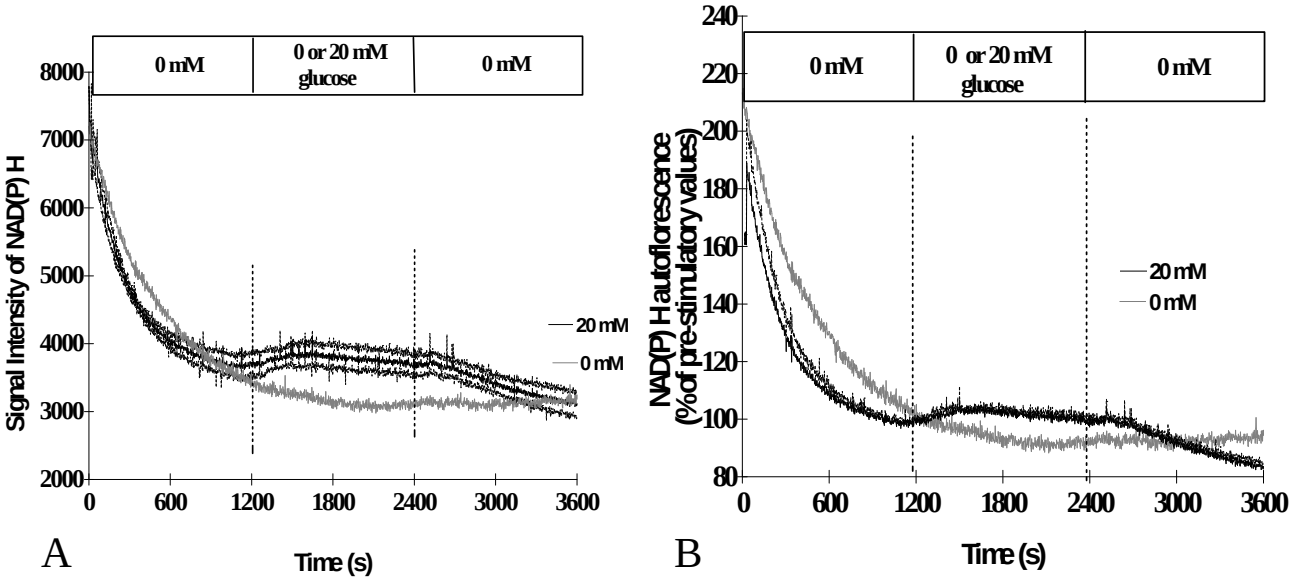


Figure 4.25 Changes in NAD(P)H signal in response to 20 mM glucose in SUR1 KO mouse islets pre-incubated with 100 µM gatifloxacin at 36 °C. A) Glucose concentration increased to 20 mM (from 1200 to 2400 seconds) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The signal intensity (arbitrary units) is plotted against time in seconds. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose. Values are means ± SEM of six experiments for each. The islets were obtained from 2 different preparations. **(B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

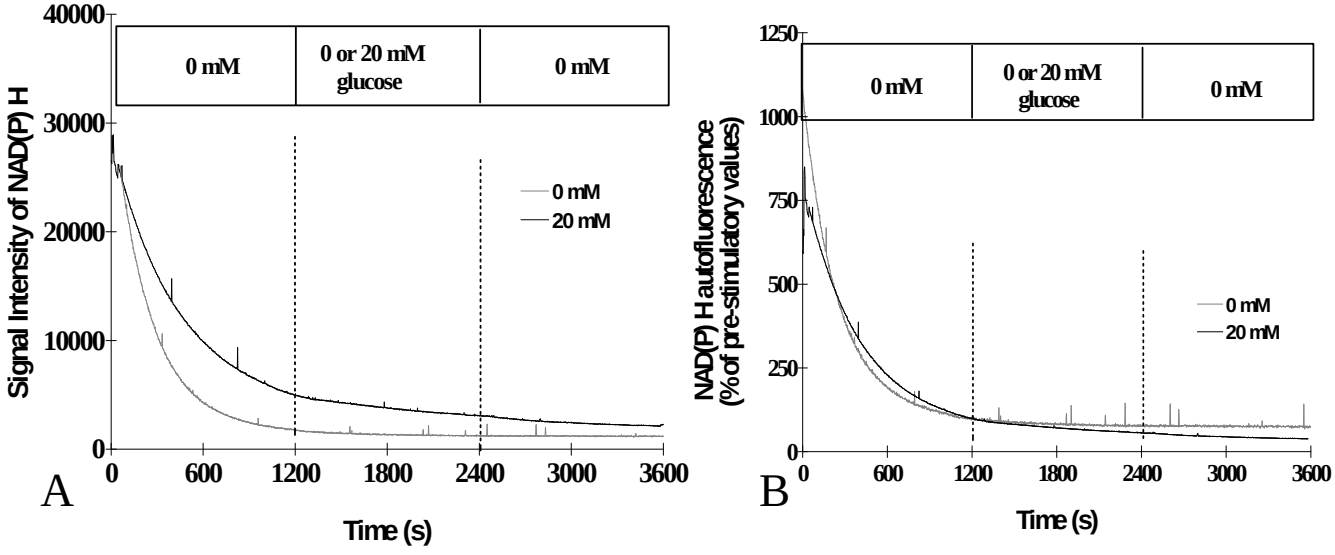


Figure 4.26 Changes in NAD(P)H signal in response to 20 mM glucose in SUR1 KO mouse islets pre-incubated with 100 µM moxifloxacin at 36 °C. A) Glucose concentration increased to 20 mM (from 1200 to 2400 seconds) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The signal intensity (arbitrary units) is plotted against time in seconds. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose. Values are means ± SEM of six experiments for each. The islets were obtained from 2 different preparations. **(B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

When the islets were preincubated with ciprofloxacin, the fluorescence started to decrease till glucose concentration 20 mM was added causing immediate increase by 30-50% of the prestimulatory values and 60 % relative to the washout experiment. The values returned to pre-stimulatory level when glucose was removed from the perifusion medium (Fig 4.27 A and B).

To sum up the results of pre-incubation protocol, the fluorescence increase was nearly unimpaired after ciprofloxacin, only modest after gatifloxacin and abolished after moxifloxacin. When compared to normal NMRI mice, it is clear that the inhibition of mitochondrial energetics by these fluoroquinolones is not secondary to a block of plasma membrane K_{ATP} channels but rather a purely primary effect.

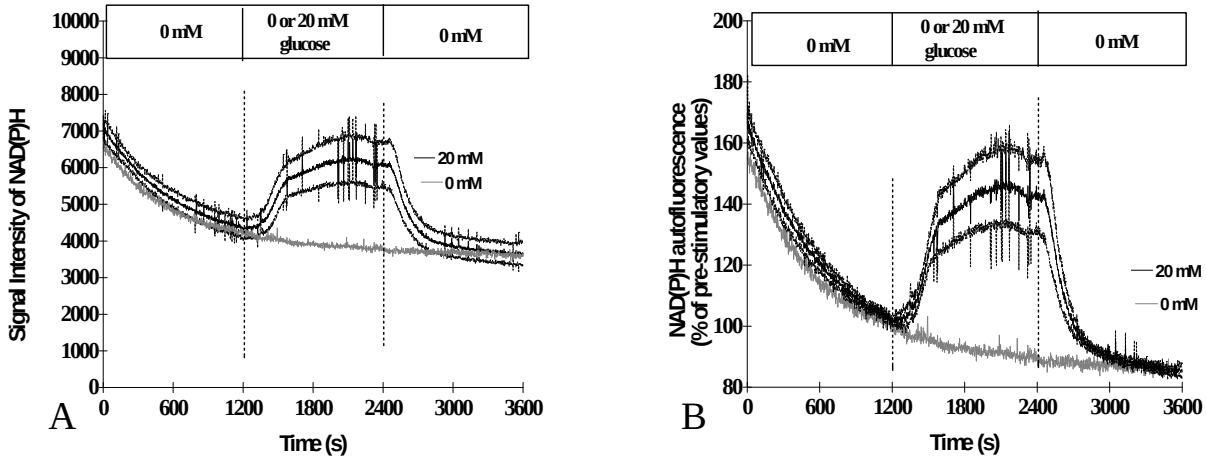


Figure 4.27 Changes in NAD(P)H signal in response to 20 mM glucose in SUR1KO mouse islets pre-incubated with 100 µM ciprofloxacin at 36 °C. A) Glucose concentration increased to 20 mM (from 1200 to 2400 seconds) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The signal intensity (arbitrary units) is plotted against time in seconds. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose. Values are means ± SEM of six experiments for each. The islets were obtained from 2 different preparations. **B)** The same protocol but the values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

4.2.4 FAD autofluorescence measurement in islets pre-incubated with fluoroquinolones

The same protocol for recording NAD(P)H autofluorescence was applied to measure FAD autofluorescence. Raising the glucose concentration to 20 mM resulted in a decrease by 25 % of the pre-stimulatory values and 35 % relative to the washout experiment. Although the values did not return to the prestimulatory values, they followed the level of washout experiments (Fig 4.28).

When NMRI islets were pre-incubated with gatifloxacin, increasing the glucose concentration resulted in a decrease of the intensity by 26 % which was closely similar to the control experiment. Compared to the washout experiment, the net decrease was 38 %. When glucose was removed, the intensity showed a modest increase reaching to

75% of the prestimulatory values compared to 81 % for the washout experiment (Fig 4.29).

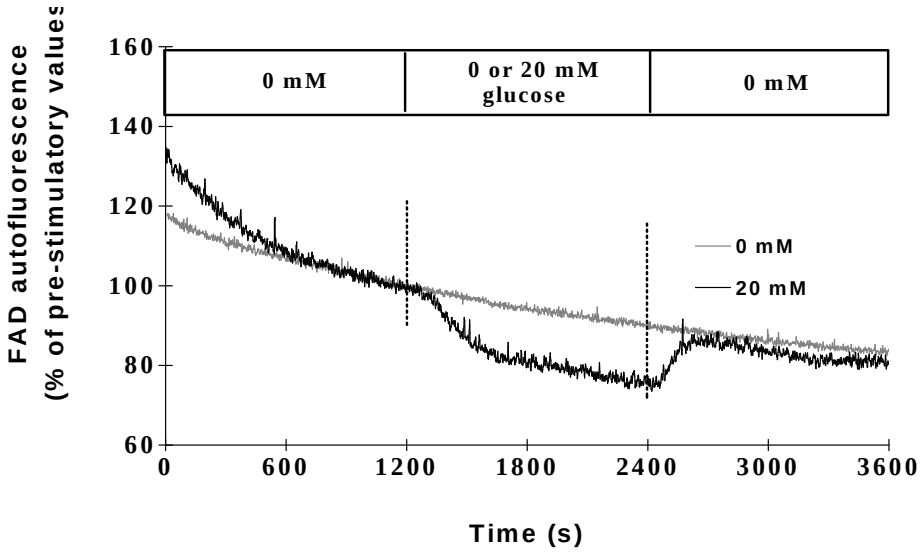


Figure 4.28 Changes in FAD signal in response to 20 mM glucose in NMRI mouse islets at 36 °C. Islets pre-incubated for one hour with Krebs-Ringer medium (5 mM glucose). A decrease in fluorescence reflects an increase in the reduced state of flavins on exposure to 20 mM glucose which is reversible when glucose is no longer present in the medium. This was compared to perifusion the islets with only Krebs-Ringer medium with no glucose for the whole one hour (the grey line). Values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

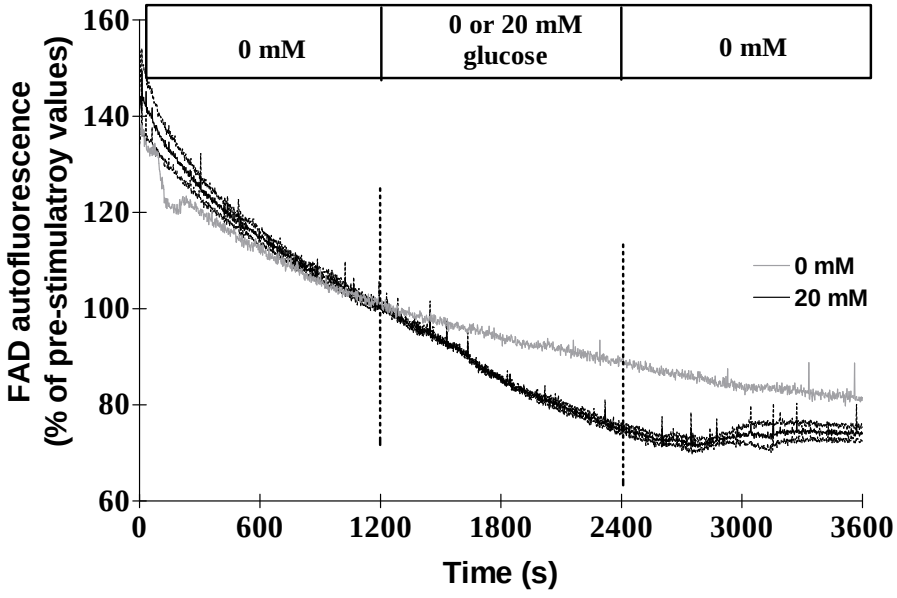


Figure 4.29 Changes in FAD signal in response to 20 mM glucose in NMRI mouse islets pre-incubated with 100 µM gatifloxacin at 36 °C. Glucose concentration increased to 20 mM (from 1200 to 2400 seconds) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose. Values are means ± SEM of six experiments for each. The islets were obtained from 2 different preparations. Values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

When NMRI islets were pre-incubated with moxifloxacin, 20 mM glucose caused the intensity to slowly decrease by 20 % of the pre-stimulatory values and 5 % relative to the washout experiment. The intensities then followed similar kinetic profile for the rest of perifusion (Fig 4.30).

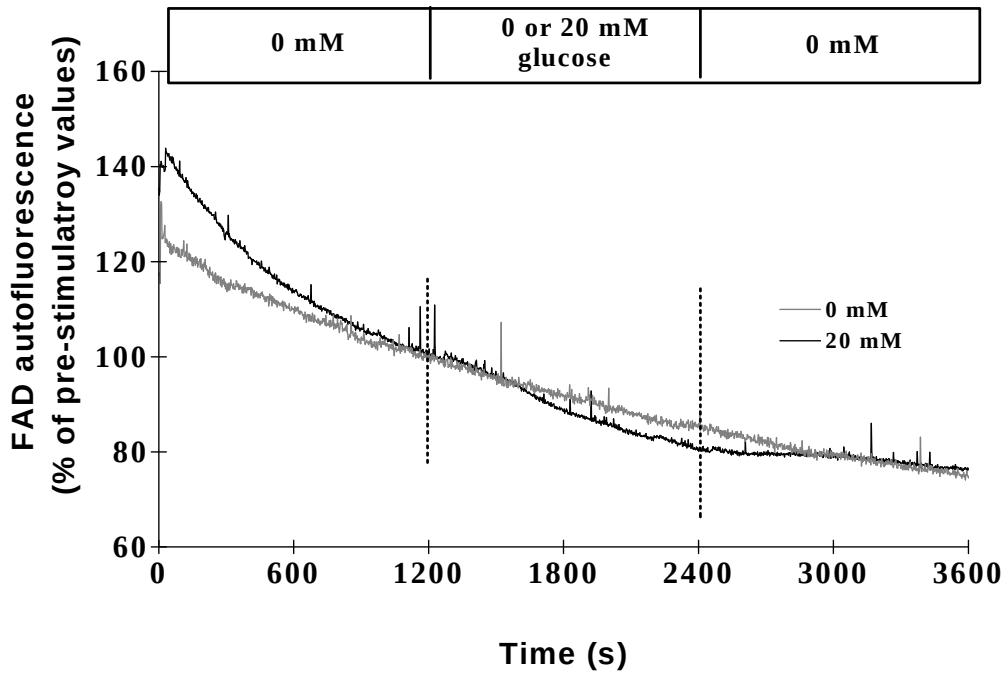


Figure 4.30 Changes in FAD signal in response to 20 mM glucose in NMRI mouse islets pre-incubated with 100 µM moxifloxacin at 36 °C. Glucose concentration increased to 20 mM (from 1200 to 2400 seconds) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose. Values are means ± SEM of six experiments for each. The islets were obtained from 2 different preparations. Values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

When NMRI islets were pre-incubated with ciprofloxacin, the decrease caused by perifusion with 20 mM glucose was 20 % of the prestimulatory values and 30 % compared to washout experiment (Fig 4.31).

To sum up, the typical reaction to 20 mM glucose was sluggish but visible in each case. There were clear differences between the effect size after exposure to gatifloxacin, which was nearly unimpaired while after exposure to ciprofloxacin was clearly reduced and that after exposure to moxifloxacin, which was no longer significant

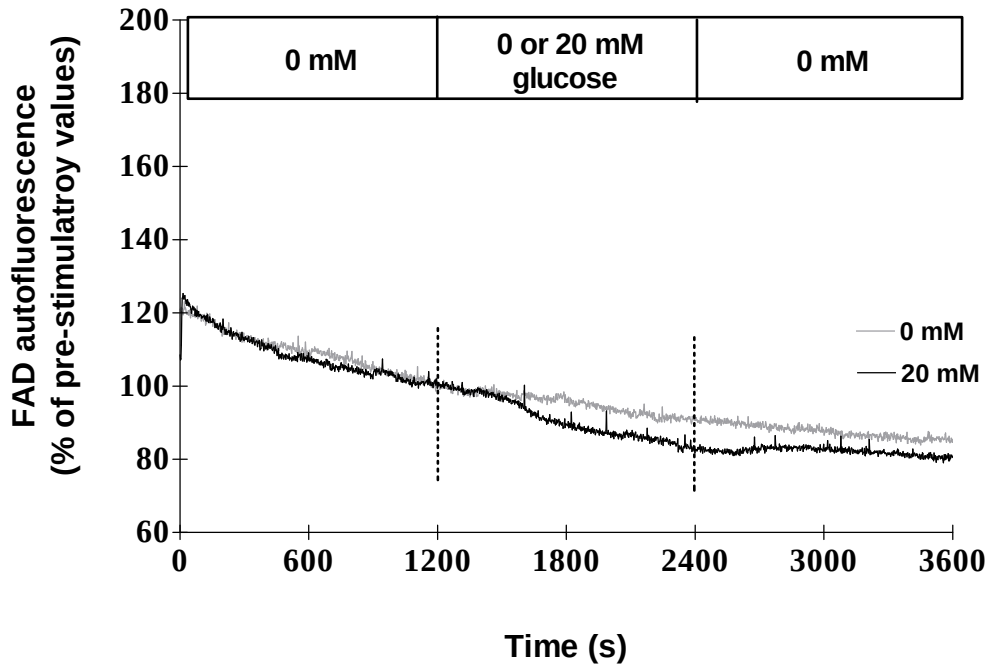


Figure 4.31 Changes in FAD signal in response to 20 mM glucose in NMRI mouse islets pre-incubated with 100 µM ciprofloxacin at 36 °C. Glucose concentration increased to 20 mM (from 1200 to 2400 seconds) and from 2400 seconds till the end again lowering the glucose concentration to 0 mM. The grey line shows the effect of perifusion of the islets with only Krebs-Ringer solution without glucose. Values are means ± SEM of six experiments for each. The islets were obtained from 2 different preparations. Values are normalized by setting the last pre-stimulatory value (20 min) to 100%.

4.3 Measurement of ΔΨm

The inhibitory effect on the glucose-induced generation of reducing equivalents raised the question whether the fluoroquinolones affect the mitochondrial membrane potential. To address this possibility, the mitochondrial membrane potential was assessed using Rh123 (10 µM). By monitoring Rh123 fluorescence levels over time, changes in ΔΨm were ascertained in response to changes in metabolic fuel availability and then after the addition of metabolic inhibitors. The overall cellular fluorescence increases while the dye concentration inside mitochondria decreases and so loaded cells show a marked increase in fluorescence upon mitochondrial depolarization.

When the β cells of NMRI mice were perifused with 5 mM glucose, Rh123 fluorescence was stable from minutes 20-60 of perifusion with no noticeable photobleaching. The intensity was then markedly increased by 15 ± 5 % in response to 10 mM NaN_3, which is consistent with a reduction or dissipation of ΔΨm, resulting in more visible dye in the cytosol (Fig 4.32). Similar results were obtained with 5 µM CCCP which is a powerful mitochondrial uncoupler that specifically increases the proton permeability of the inner mitochondrial membrane. In contrast with the uncoupler CCCP, the effect of NaN_3 was completely reversible. These findings

confirm those of previous studies (Krippeit-Drews et al., 2000; Kindmark et al., 2001) and support the validity of the technique to measure the metabolic activity of mitochondria.

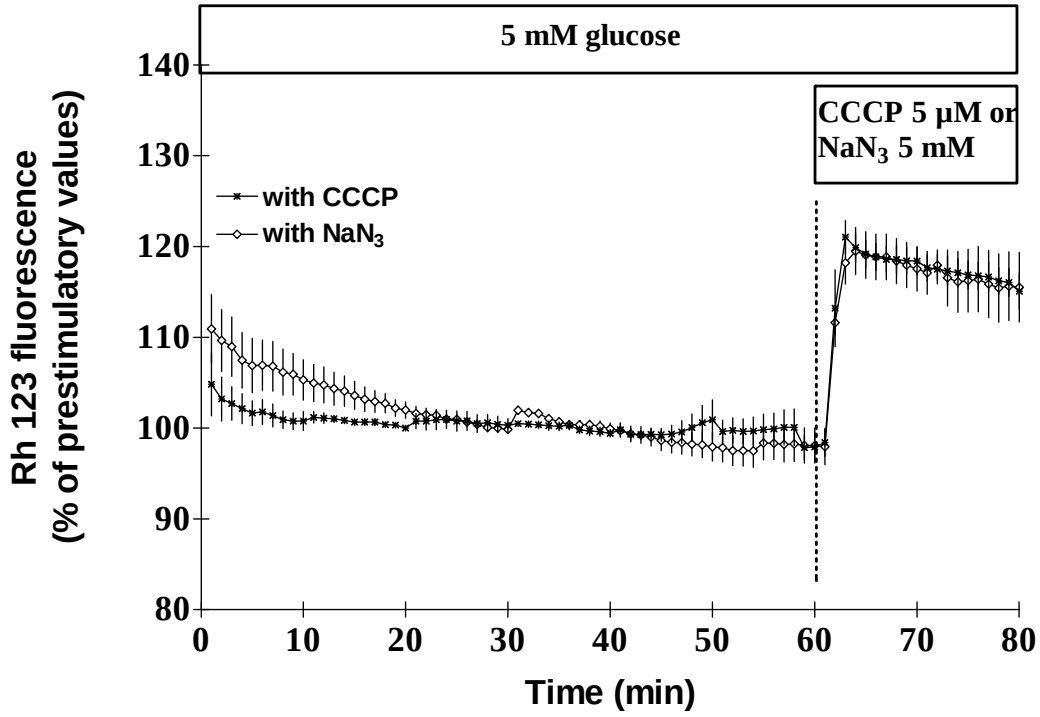


Figure 4.32 Changes in mitochondrial membrane potential in the presence of a basal glucose concentration in NMRI β cells. The cells were exposed to Krebs- Ringer medium containing 5 mM glucose for one hour showing a steady state from min. 20 onward. Addition of CCCP 5 µM or Na N_3 5 mM for 20 minutes resulted in increased rhodamine-123 fluorescence, indicating depolarization of ΔΨm reaching up to 20 %. Results are means ± S.E.M. of results of 20 β cells each from six experiments from two different preparations. Values were normalized to 100% at minute 20.

4.3.1 Measurement of ΔΨm with direct perifusion with fluoroquinolones

In the following set of experiments, NMRI β cells were perifused with 5 mM glucose, then exposed to the fluoroquinolones for 30 minutes, followed by washout for 10 minutes. Lastly either CCCP or NaN3 were applied to confirm the mitochondrial origin of the signal.

Addition of moxifloxacin to 5 mM glucose resulted immediately in a decrease of the fluorescence intensity by 5 % with a steady state reached after 4 minutes (Fig 4.33). Furthermore, the responses of the β cells to the metabolic inhibitors were not alike. While the fluorescence increase in response to azide was similar to the control (20% and 25 % respectively), the modest depolarization (10%) produced by CCCP did not continue for more than 3 minutes then returned rapidly to 100 % of pre-exposure to moxifloxacin (Fig 4.34).

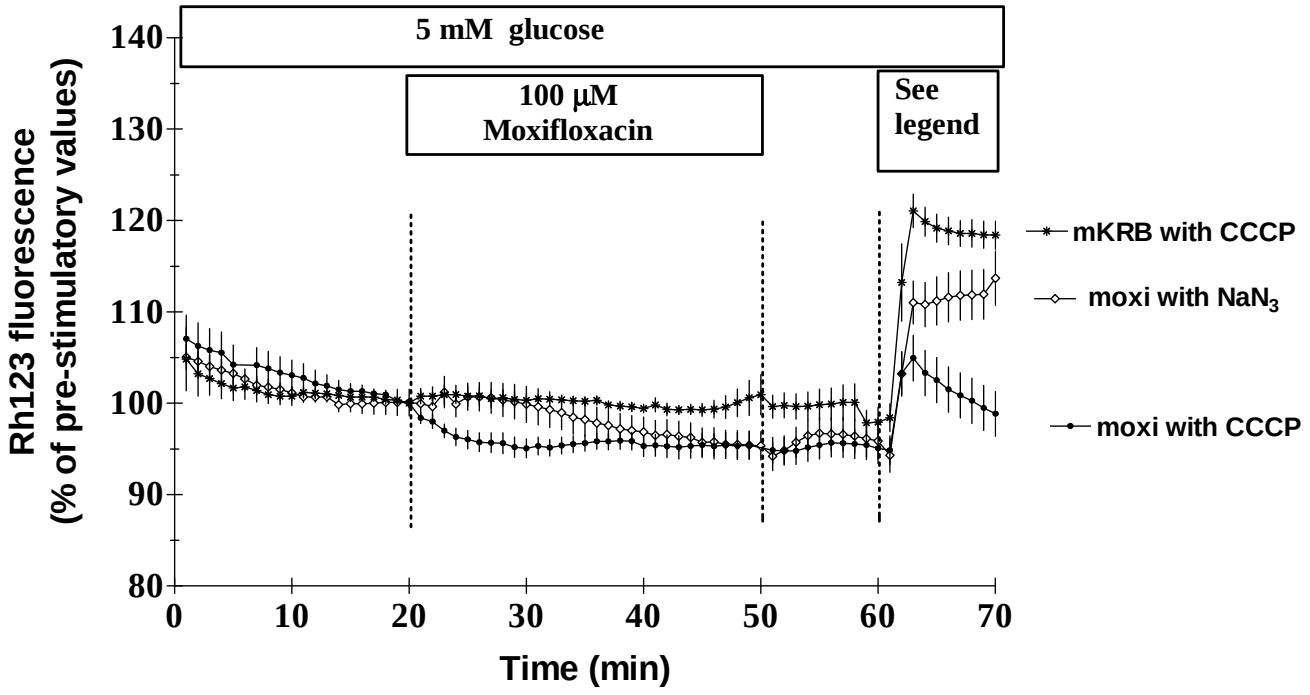


Figure 4.33 Effect of moxifloxacin on mitochondrial membrane potential in the presence of a basal glucose concentration in NMRI β cells. Krebs-Ringer medium containing 5 mM glucose throughout the experiments, while Moxifloxacin 100 µM were added from minute 20 for half an hour then washed out from minute 50-60 then CCCP 5 µM or 5 mM NaN_3 added for 20 minutes. Moxifloxacin is associated with hyperpolarization immediately when added to the medium reaching steady state after 6 minutes. The response of the β cells to the uncoupler was increased by 20% but not last for more than two minutes then drops again, while response to azide was not affected. Values were normalized to 100% at minute 20.

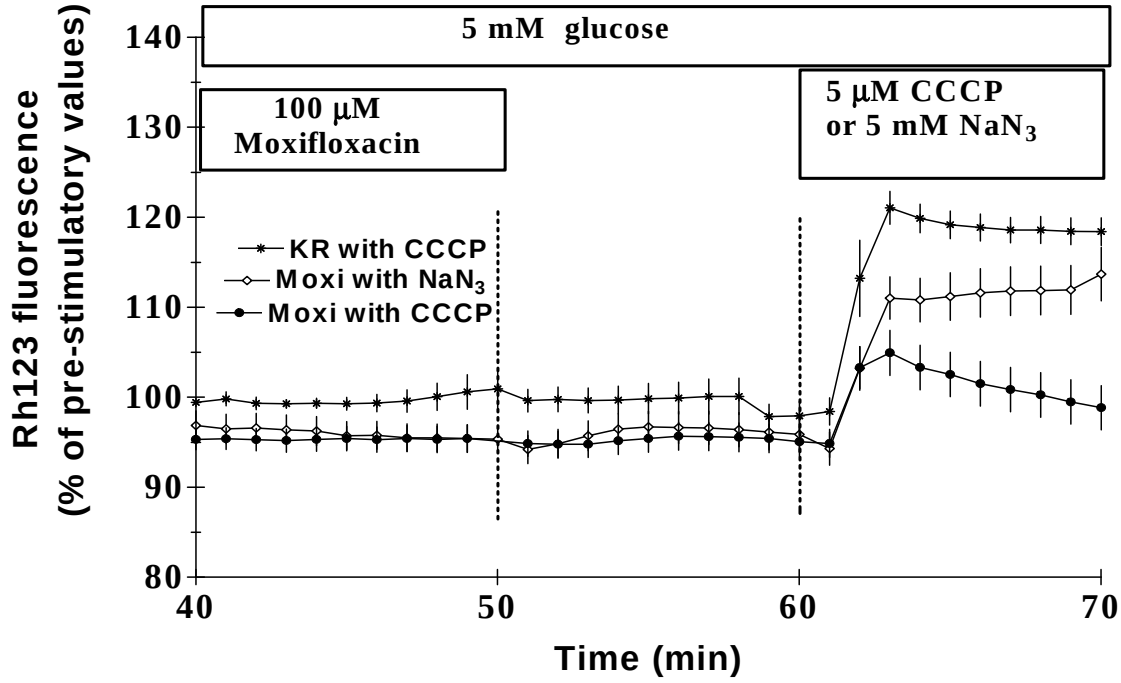


Figure 4.34 Comparison of the effect of CCCP and NaN_3 on mitochondrial membrane potential after wash out of moxifloxacin. The β cells show unsustained decrease in Rh123 fluorescence with CCCP compared to steady state with NaN_3. Values are means ± SEM of 27 and 16 β cells respectively. Values were normalized to 100% at minute 20.

In case of ciprofloxacin, the Rh123 intensity decreased only by 4 % and returned to the pre-stimulatory values with washout. Further depolarization by CCCP (increase by 15 ± 5 %) was similar to that of the control experiment (Fig 4.35).

In case of gatifloxacin, it caused only a decrease of Rh123 fluorescence by 6 % but not affected by washout for 10 minutes. Further perifusion with CCCP increased the fluorescence by 25 % (Fig 4.36).

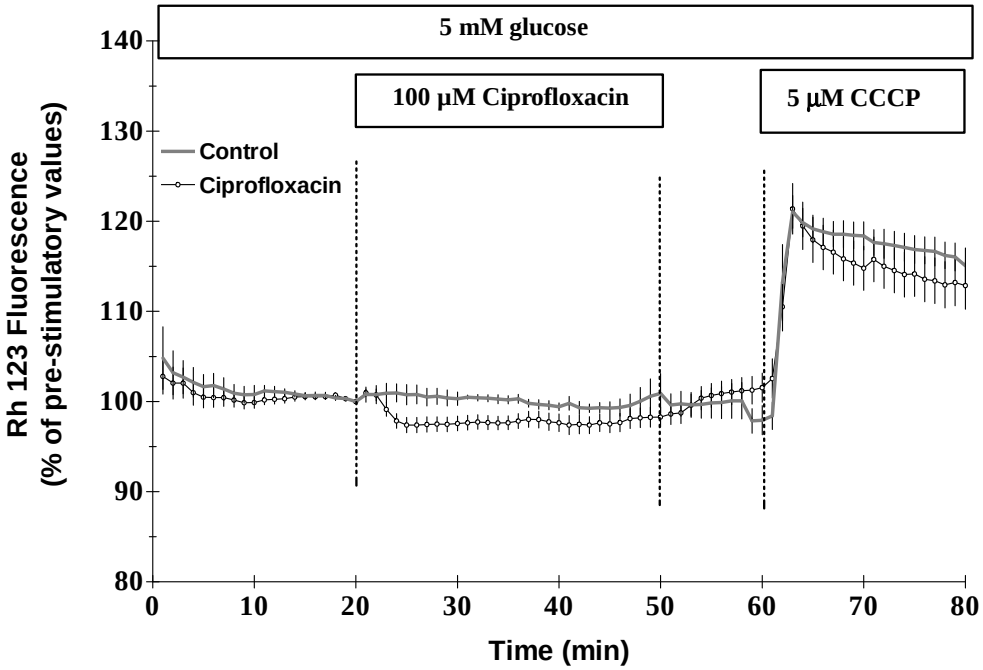


Figure 4.35 Effect of ciprofloxacin on mitochondrial membrane potential in the presence of a basal glucose concentration in NMRI β cells. Krebs-Ringer medium containing 5 mM glucose was used throughout the experiments. Ciprofloxacin 100 µM were added from minute 20 for half an hour then washed out from minute 50-60 then CCCP 5 µM added from minute 60-80. The response of the β cells appeared more or less the same as the control cells regarding exposure to ciprofloxacin and post wash out perifusion with the uncoupling agent. Values are means ± SEM of 27 β cells. Values were normalized to 100% at minute 20.

When the three drugs were compared concerning the effect on Rh123 fluorescence as well as the uncoupler CCCP, no significant differences was present between ciprofloxacin and control experiment. Moxifloxacin produced hyperpolarizing effect that persisted after washout and even dampened the uncoupler effect while it was without any influence on the azide induced-depolarization. The mild hyperpolarizing effect produced by gatifloxacin was not remarkable as with moxifloxacin (Fig 4.37).

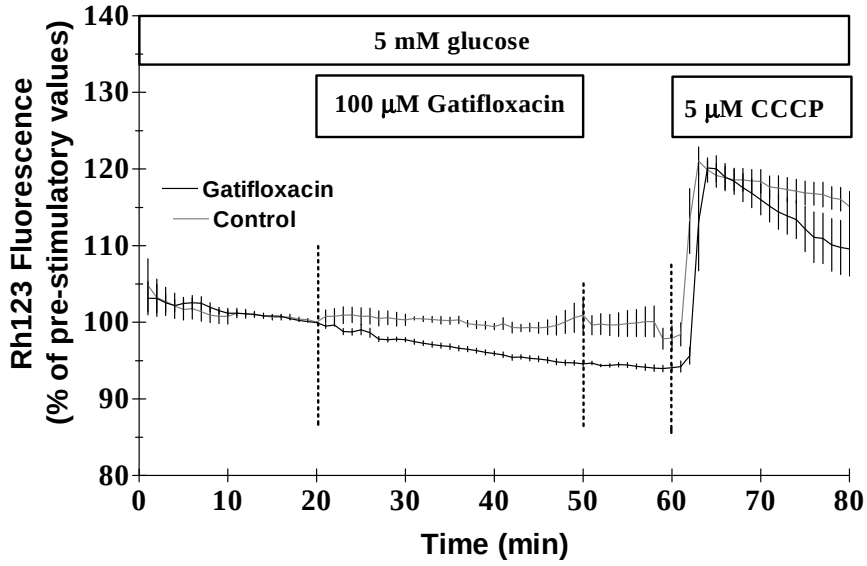


Figure 4.36 Effect of gatifloxacin on mitochondrial membrane potential in the presence of a basal glucose concentration in NMRI β cells. Krebs-Ringer medium containing 5 mM glucose was used throughout the experiments. Gatifloxacin 100 µM were added from minute 20 for half an hour then washed out from minute 50-60 then CCCP 5 µM added for 20 minute. The response of the β cells appeared more or less the same as the control cells regarding exposure to gatifloxacin (except for small decrease in Rh123 fluorescence) and post wash out perifusion with the uncoupling agent. Values are means ± SEM of 32 β cells. Values were normalized to 100% at minute 20.

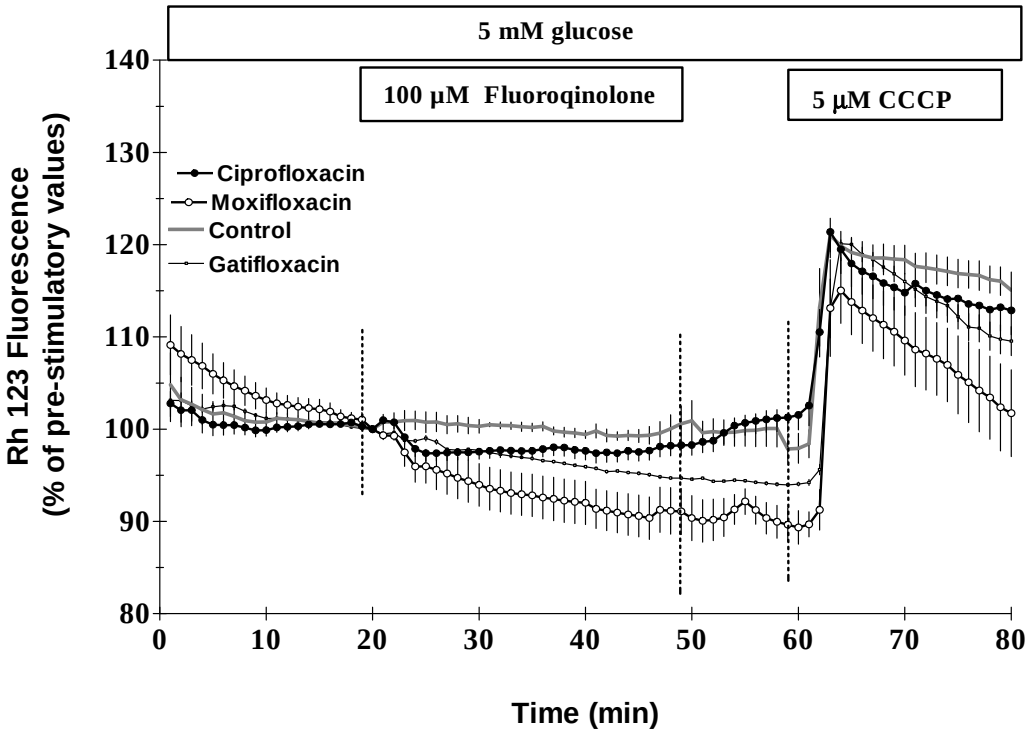


Figure 4.37 Effect of the three fluoroquinolones on mitochondrial membrane potential in the presence of a basal glucose concentration in NMRI β cells. In the control experiments, the cells were perifused with mKRB 5 mM glucose for 1 hour, then CCCP was added from minute 60-80. Values were normalized to 100% at minute 20.

4.3.2 Changes of ΔΨm as a result of pre-incubation with fluoroquinolones

To determine the effect of fluoroquinolones on glucose-induced changes in mitochondrial membrane potential, β cells were pre-incubated with 100 µM of each of the three compounds for 1 hour and then the effect of perifusion with 20 mM glucose on Rh123 fluorescence was examined. In control experiments, the hyperpolarization produced by the increase in glucose concentration to 20 mM was monitored followed by 0 mM glucose again for 20 minutes to test for reversibility. As shown in (Fig 4.38), raising the glucose concentration to 20 mM resulted in mitochondrial hyperpolarization as evidenced by the average decrease by 25 ± 5 % in Rh123 fluorescence. When glucose was removed from the perfusion medium, fluorescence immediately started to return to 90% which attained also by washout experiment.

In case of gatifloxacin, increase in glucose concentration resulted in a decrease in Rh123 fluorescence by 32-34 % after a lag of 3 minutes. The values returned to the pre-stimulatory level when glucose was removed from the medium. The washout experiment showed a slight decrease of fluorescence by 5 % after 25 minutes (Fig 4.39).

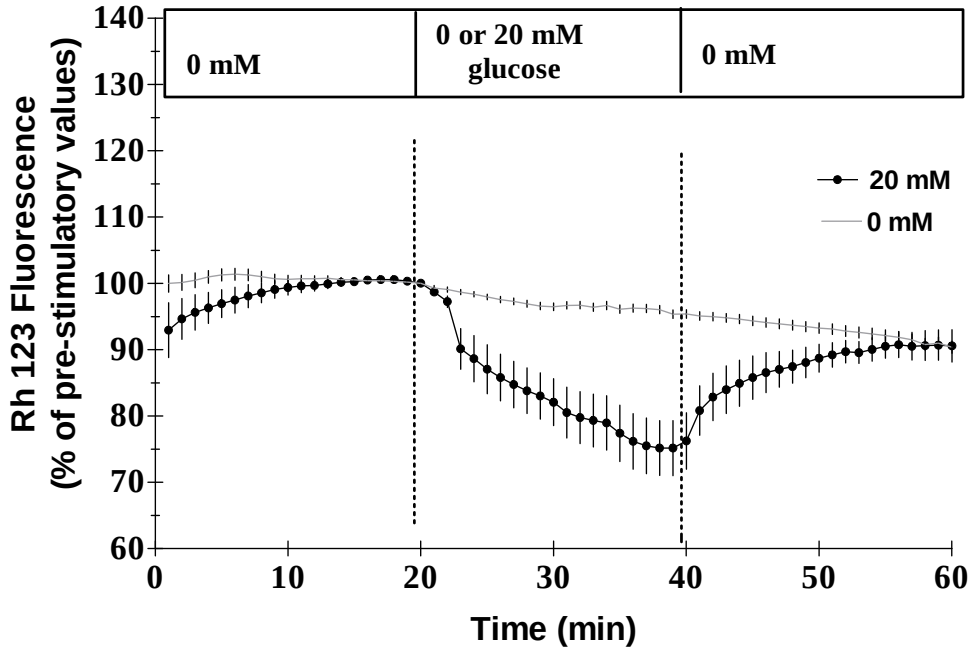


Figure 4.38 Effect of pre-incubation with mKRB 5 mM glucose on mitochondrial membrane potential in β cells exposed to glucose 20 mM. The cells were perifused with Krebs-Ringer medium containing 0 mM glucose for 20 minutes. The signal intensity of Rh123 started to decrease in response to increased glucose concentration to 20 mM from for 20 minutes. The effect is compared with wash out i.e. exposure of the cells to KR containing 0 mM during the whole 60 minutes protocol (the grey line). Values are means ± SEM of 3 experiments for each (19 β cells). Values were normalized to 100% at minute 20.

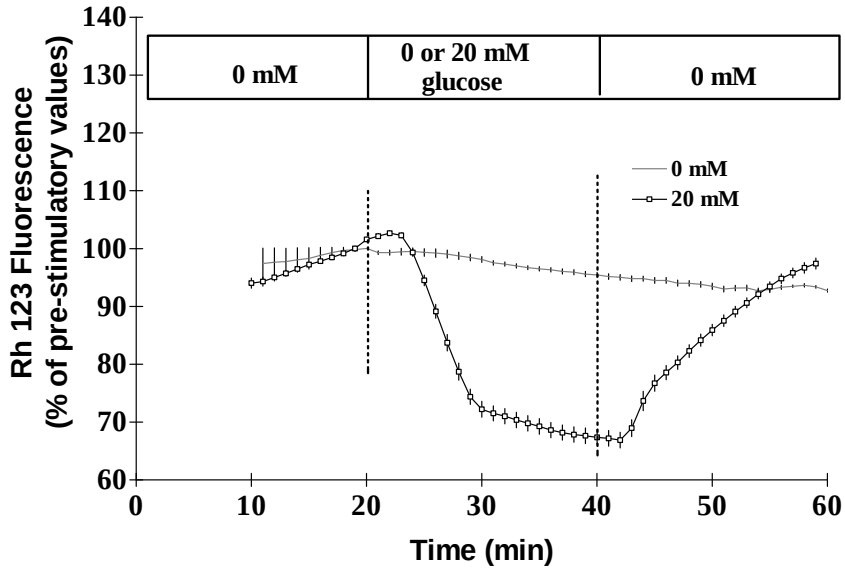


Figure 4.39 Effect of pre-incubation with gatifloxacin on mitochondrial membrane potential in β cells exposed to glucose 20 mM. The cells were perifused with Krebs-Ringer medium containing 0 mM glucose for 20 minutes. The signal intensity of Rh123 started to decrease in response to glucose concentration 20 mM for 20 minutes to reach steady state at minute 33. The effect is compared with wash out of gatifloxacin i.e. exposure of the cells to KR containing 0 mM during the whole 60 minutes protocol (the grey line). Values are means ± SEM of 3 experiments for each (25 β cells). Values were normalized to 100% at minute 20.

In case of ciprofloxacin, fluorescence was stable till glucose 20 mM was applied causing slowly progressive decrease of the fluorescence by 20-25 % of the pre-stimulatory values. This was followed by an incomplete return of the values to 88 % of the pre-stimulatory level when glucose was removed from the medium. The washout experiment showed slowly reduced fluorescence values by 8 % (Fig 4.40).

In case of moxifloxacin, increase in glucose concentration was followed by a decrease in Rh123 fluorescence by 32-35 % after a lag of 4 minutes. The values returned to 96 % of the pre-stimulatory level when glucose was removed from the medium. The washout experiment showed reduced fluorescence values by 4 % after minute 40 (Fig 4.41).

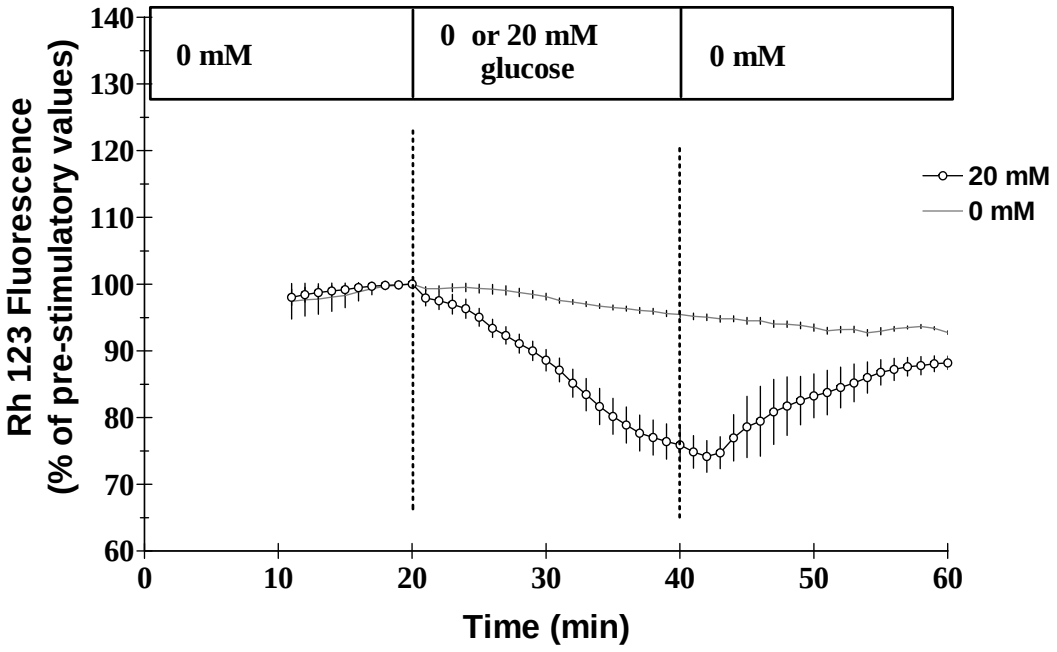


Figure 4.40 Effect of pre-incubation with ciprofloxacin on mitochondrial membrane potential in β cells exposed to glucose 20 mM. The cells were perifused with Krebs-Ringer medium containing 0 mM glucose for 20 minutes. The signal intensity of Rh123 started to reversibly decrease in response to increased glucose concentration to 20 mM for 20 minutes .The effect is compared with wash out of ciprofloxacin i.e. exposure of the cells to KR containing 0 mM during the whole 60 minutes protocol (the grey line) .Values are means ± SEM of 3 experiments for each (22 β cells). Values were normalized to 100% at minute 20.

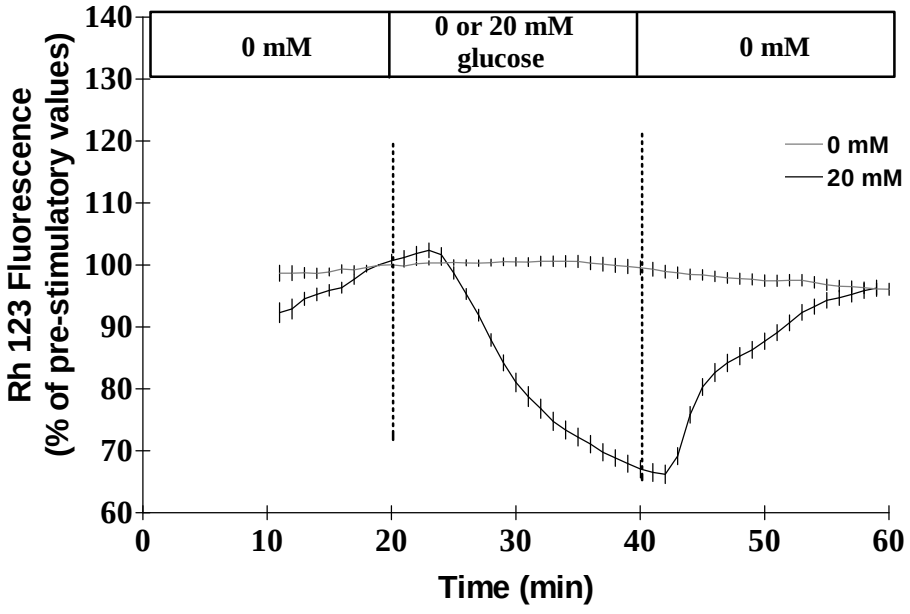


Figure 4.41 Effect of pre-incubation with moxifloxacin on mitochondrial membrane potential in β cells exposed to glucose 20 mM. The cells were perifused with Krebs-Ringer medium containing 0 mM glucose for 20 minutes. The signal intensity Rh123 started to reversibly decrease in response to increased glucose concentration to 20 mM for 20 minutes .The effect is compared with wash out of moxifloxacin i.e. exposure of the cells to KR containing 0 mM during the whole 60 minutes protocol (the grey line) .Values are means ± SEM of 3 experiments for each (31 β cells). Values were normalized to 100% at minute 20.

Comparison between pre-incubation of the three drugs on the Rh123 fluorescence was shown in (Fig 4.42). It seems that fluoroquinolones had a moderate hyperpolarizing effect of their own with varying degrees with the rank order: gatifloxacin and moxifloxacin > ciprofloxacin.

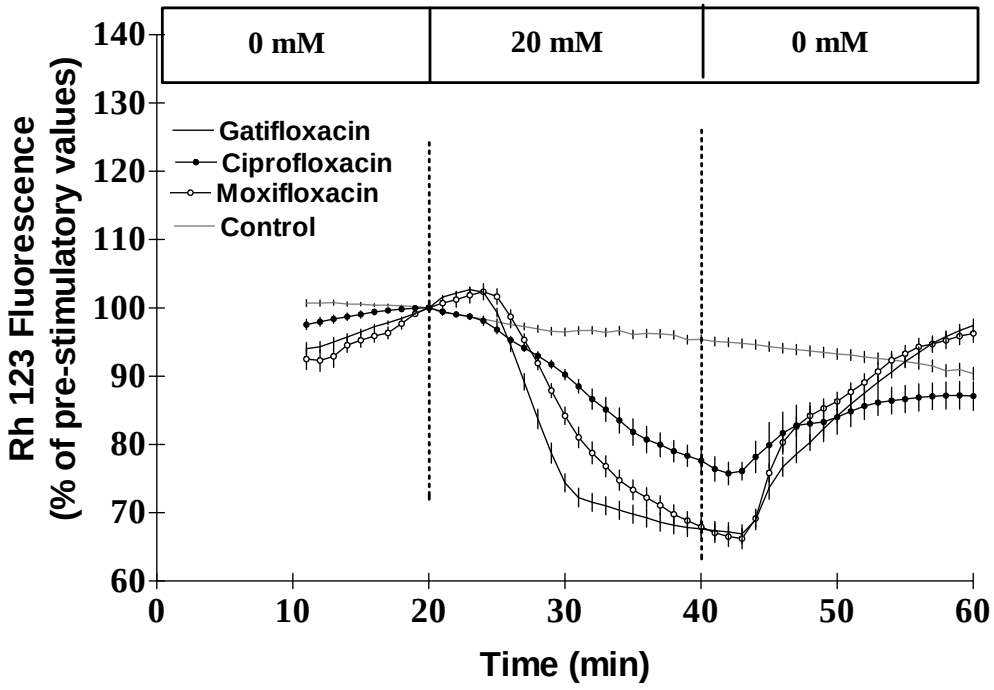


Figure 4.42 Effect of pre-incubation with each fluoroquinolone on mitochondrial membrane potential in β cells exposed to glucose 20 mM. Comparison of the 3 compounds with the control experiments in which β cells were only perifused with mKRB 0 mM glucose for one hour. Values were normalized to 100% at minute 20.

4.3.3 Changes of ΔΨm on direct exposure to fluoroquinolones with stimulatory glucose

In this set of experiments, β cells were exposed to 20 mM glucose for 20 minutes and then with such stimulatory glucose, fluoroquinolones were added. As seen in (Fig 4.43), moxifloxacin was the only fluoroquinolone that reverse the depolarizing effect of 20 mM glucose reaching to 90 % of the pre-stimulatory value. This effect was similar to that when glucose 0 mM was used. On the contrary, both gatifloxacin and ciprofloxacin had not any influence on the glucose depolarizing effect.

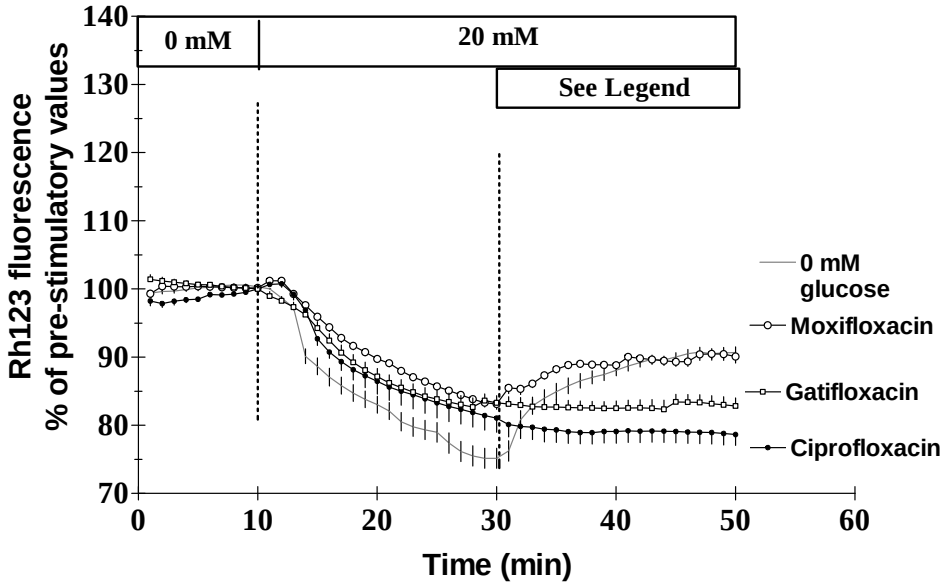


Figure 4.43 Effect of perifusion with each fluoroquinolone on mitochondrial membrane potential in β cells after being exposed to glucose 20 mM. The cells were perifused with mKRB 0 mM glucose for 10 minutes, increased to 20 mM from minute 10-30. 100 μM of each fluoroquinolone were then added from minute 30-50. In the control experiments, the cells were perifused with mKRB 0 mM from minute 30-50. Values are means ± SEM of 3 experiments for each; gatifloxacin (25), moxifloxacin (32), ciprofloxacin (34), and control (18) β cells. Values were normalized to 100% at minute 10.

4.4 ATP/ADP ratio

Because the increase in the ATP-to-ADP ratio is fundamental for the initiation of β cell electrical activity and insulin secretion, the cytosolic adenine nucleotide levels and ADP/ATP ratio were examined. These measurements were performed on statically incubated islets, where the conditions were chosen to fit to pre-incubation scheme i.e. the islets were incubated for 1 h in the presence of 100 μM of the fluoroquinolones, then the medium was replaced and after 20 min the glucose concentration was raised from 0 to 20 min as shown in (Fig 4.44). At the end of the 20 min exposure to high glucose, the adenine nucleotide content was measured. Only moxifloxacin, but neither gatifloxacin nor ciprofloxacin significantly affected the ATP/ADP ratio. The reduction of the ratio was of moderate extent (down to 81.4 $\pm$ 3.3 %), but was highly significant ($p = 0.0078$, Wilcoxon signed rank test). For comparison, the uncoupler CCCP (5 μM) reduced the ATP/ADP ratio to 43 %. In the presence of 5 mM glucose, the acute addition of the fluoroquinolones was without effect on the ATP/ADP ratio (Fig 4.45).

Since the inability of 100 μM of the fluoroquinolones to depolarize the beta cells in the perforated patch configuration (in spite of the depolarizing effect on open cell preparations) had been found when the glucose concentration in the cell bath was 5 mM, the immediate effect of fluoroquinolones on the adenine nucleotide content was

determined. To make the experimental situation comparable to the electrophysiological measurements, the exposure time was limited to 30 min. However, it turned out that none of three compounds significantly reduced adenine nucleotide content or the ATP/ADP ratio.

To assess the dependency of the above-described effect of moxifloxacin on the glucose concentration in the medium or, more generally, the energization of the beta cells, the effect of 100 µM moxifloxacin on the adenine nucleotide content was determined in the presence of 0 mM glucose and in the presence of 5 mM glucose plus 5 µM of the uncoupler of oxidative phosphorylation, CCCP. Here, the exposure time was 20 min to be comparable to experiments in which the mitochondrial membrane potential was determined (see Figure 4.33). The absence of glucose was clearly recognizable by the diminished ATP content (compare Figure 4.45), which was further reduced by CCCP. However, there was no significant change by the presence of moxifloxacin. The 50% reduction of the ATP-to-ADP ratio in the combined presence of moxifloxacin and CCCP corresponded to the effect of CCCP alone in earlier experiments of the group.

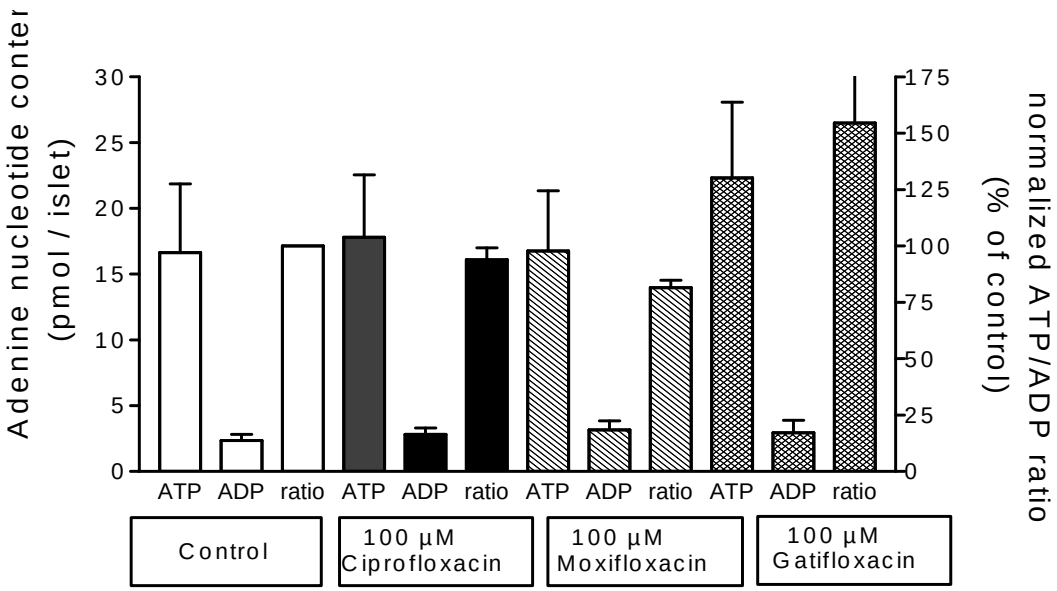


Figure 4.44 Effect of 100 µM of gatifloxacin, ciprofloxacin or moxifloxacin on the adenine nucleotide content and the ATP-to-ADP ratio as established by a 20 min exposure to 20 mM glucose. Freshly isolated islets were statically incubated for 1 h in KR medium containing 5 mM glucose and the respective fluoroquinolone. Then, the KR medium was replaced by one containing 0 mM glucose and after 20 min the glucose concentration was raised to 20 mM and the incubation was continued for another 20 min. Thereafter the incubation was stopped and the adenine nucleotide content was determined. The ratio value of each single control experiment was normalized to 100%. The data are means ± SEM of 7 experiments.

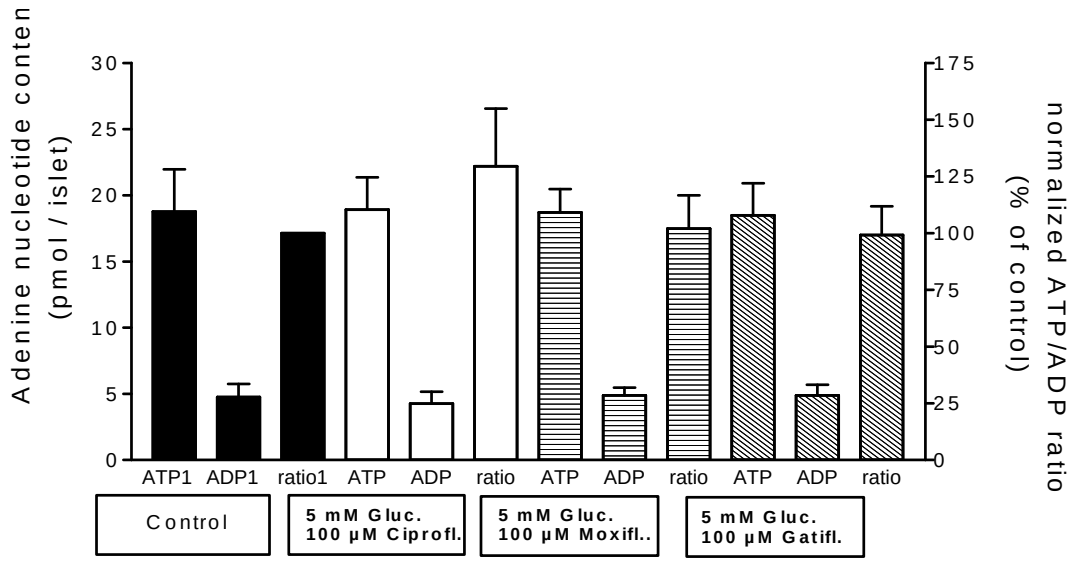


Figure 4.45 Effect of 100 µM of gatifloxacin, ciprofloxacin or moxifloxacin on the adenine nucleotide content and the ATP-to-ADP ratio in the presence of 5 mM glucose. Freshly isolated islets were statically incubated for 20 min in KR medium containing 5 mM glucose, then the respective fluoroquinolone was added and the incubation was continued for another 30 min. Thereafter the incubation was stopped and the adenine nucleotide content was determined. The ratio value of each single control experiment was normalized to 100%. The data are means ± SEM of 8 experiments.

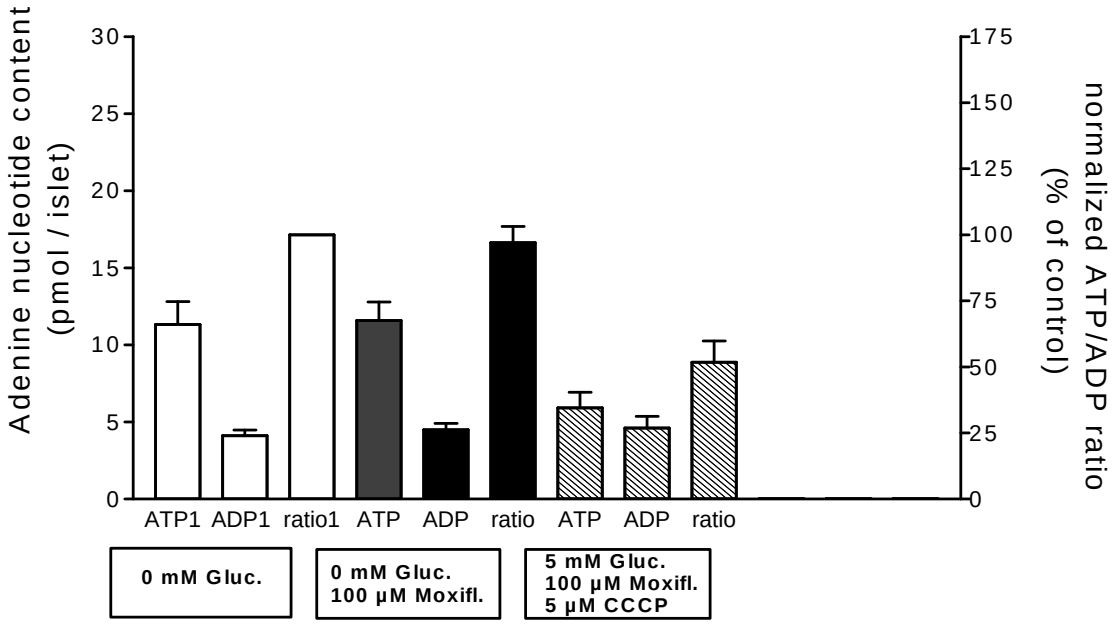


Figure 4.46 Effect of 100 µM of moxifloxacin on the adenine nucleotide content and the ATP-to-ADP ratio in the presence of 0 mM glucose or 5 mM glucose plus uncoupler. Freshly isolated islets were statically incubated for 20 min in KR medium containing either 0 mM glucose or 5 mM glucose plus 5 µM CCCP. Moxifloxacin was present from the beginning of the incubation. After 20 min the incubation was stopped and the adenine nucleotide content was determined. The ratio value of each single control experiment was normalized to 100%. The data are means ± SEM of 7 experiments.

4.5 Ultrastructural changes induced by preincubation with fluoroquinolones

To assess whether the functional changes in the beta cell energy metabolism, which had become visible in the microfluorometric measurements, can lead to morphological alterations, freshly isolated islets were cultured for 24 h in RPMI medium containing 5 mM glucose and one of the fluoroquinolones selected for this study. For control purpose, islets were also cultured in the absence of a fluoroquinolone. Each compound was tested at two concentrations, 10 and 100 µM. 100 µM was selected because this was the concentration mainly employed for the functional tests. 10 µM was chosen because it is within the range of peak plasma concentrations and because the long term exposure might reveal changes not visible during 120 min or 1h perifusions. The electromicrographs were evaluated with respect to the following criteria: granulation state of the beta cells, configuration of the rough endoplasmic reticulum (RER) configuration of the Golgi apparatus and mitochondrial morphology.

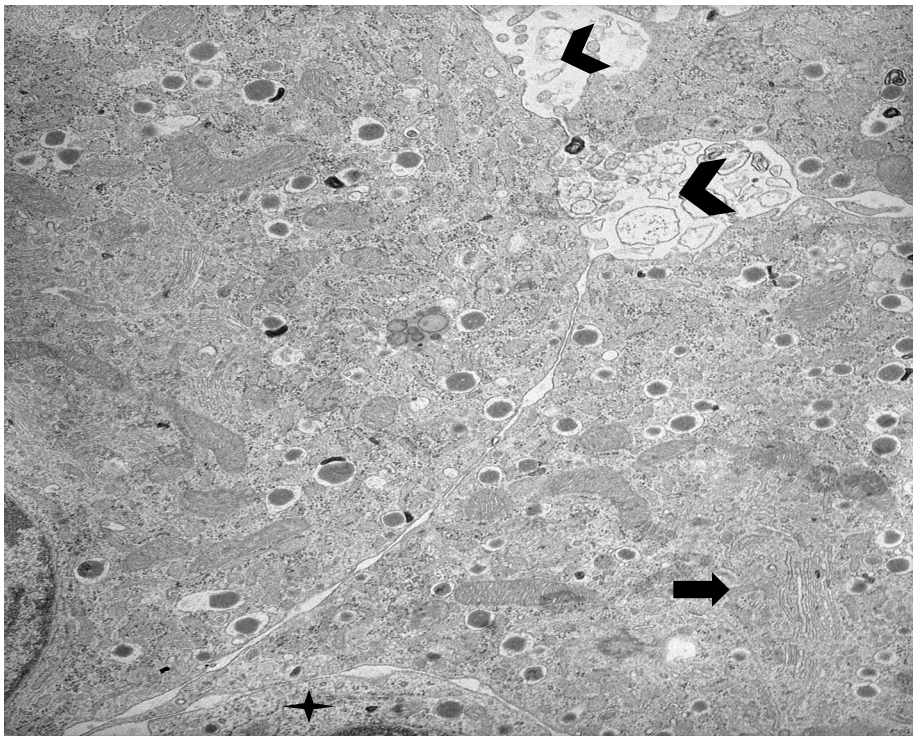
The control-cultured islets showed a well preserved morphology, in particular practically all of the beta cells within these islets showed a marked granulation. After exposure to 10 µM gatifloxacin a moderate degranulation was observed (~ 50 % of the beta cells) and the RER was elongated, but no changes were visible in the Golgi apparatus or the mitochondria (Fig 4.47A). 100 µM of gatifloxacin produced a more marked but still not a complete degranulation (~ 30% of the beta cells well granulated). The RER was now also dilated and the mitochondria showed a number of fusion- and- fission configurations (Fig 4.47B). However, there were no signs of outright mitochondrial damage of cell death.

After exposure to 10 µM ciprofloxacin at best a modest degranulation was observed (~ 70 % of the beta cells well granulated) and the RER, the Golgi apparatus and the mitochondria showed a control-like morphology (Fig 4.48A). 100 µM of ciprofloxacin however produced a strong degranulation, potentially stronger than the one by gatifloxacin (only about 10 – 15 % of the beta cells being well granulated). In contrast to gatifloxacin at the same concentration 100 µM ciprofloxacin did not affect the configuration of the RER and the Golgi apparatus. However, similar to gatifloxacin, the mitochondria showed clear changes, their size being enlarged and fusion- and –fission configurations being visible (Fig 4.48B).

After exposure to 10 µM moxifloxacin ~ 50% of beta cell was degranulated, similar to what has been observed with gatifloxacin. Also, there were no clear-cut changes in the appearance of the RER, the Golgi apparatus and the mitochondria (Fig 4.49A). After exposure to 100 µM of moxifloxacin, a degranulation was unmistakable (only about 30 % of the beta cells being well granulated). 100 µM moxifloxacin did not affect the configuration of the RER and the Golgi

apparatus. However, similar to the other fluoroquinolones the mitochondria were occasionally enlarged and fusion- and –fission configurations appeared (Fig 4.49B).

A



B

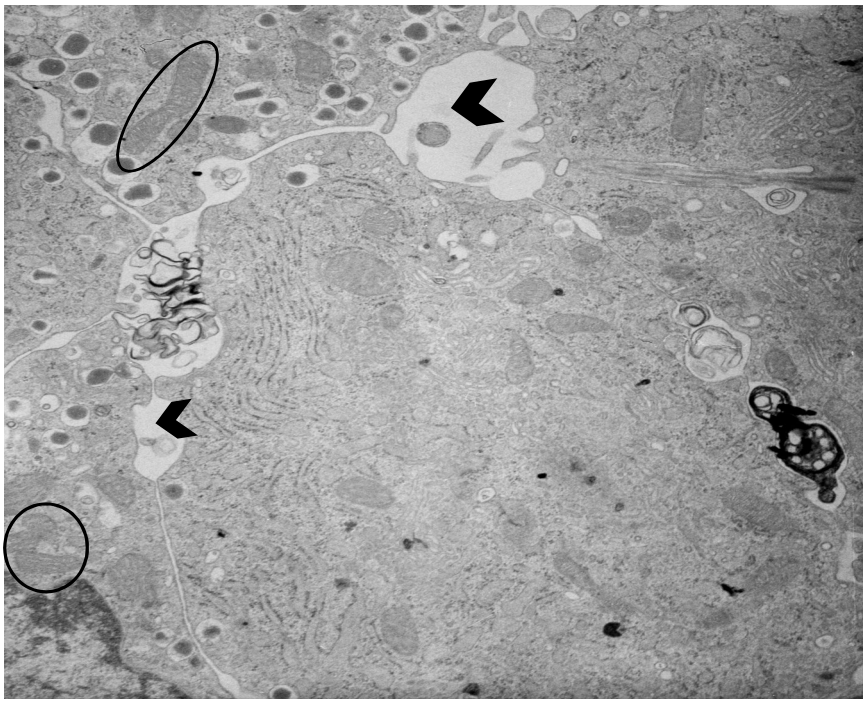
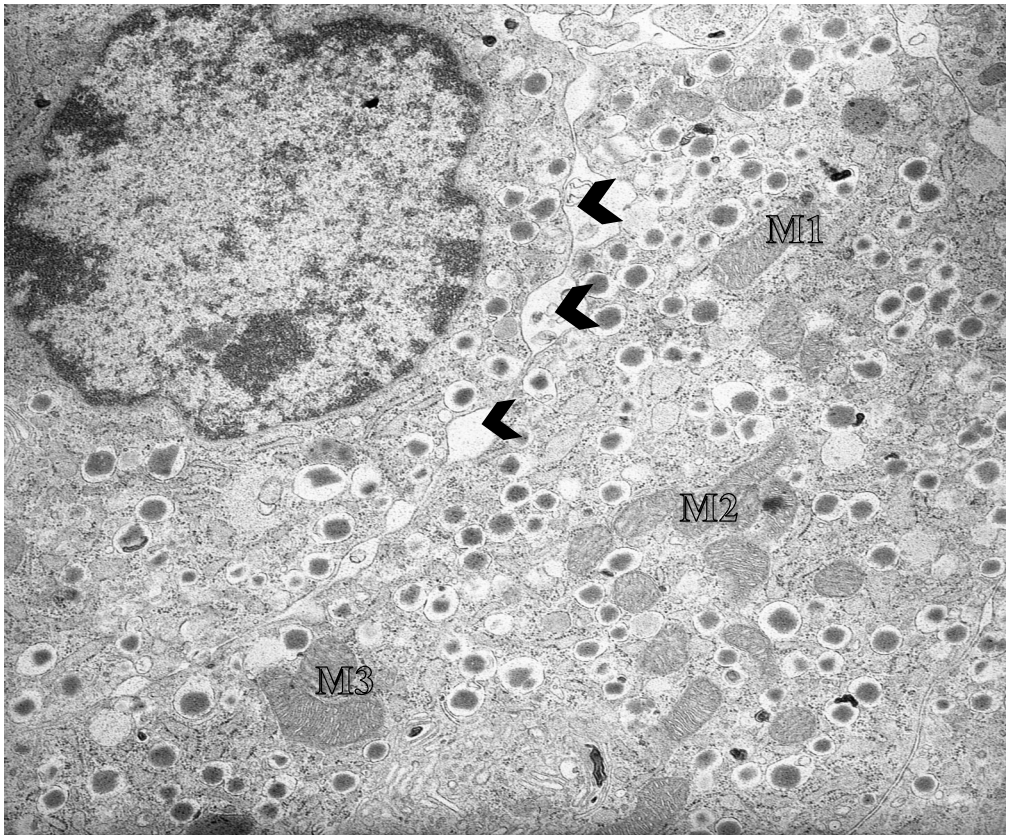
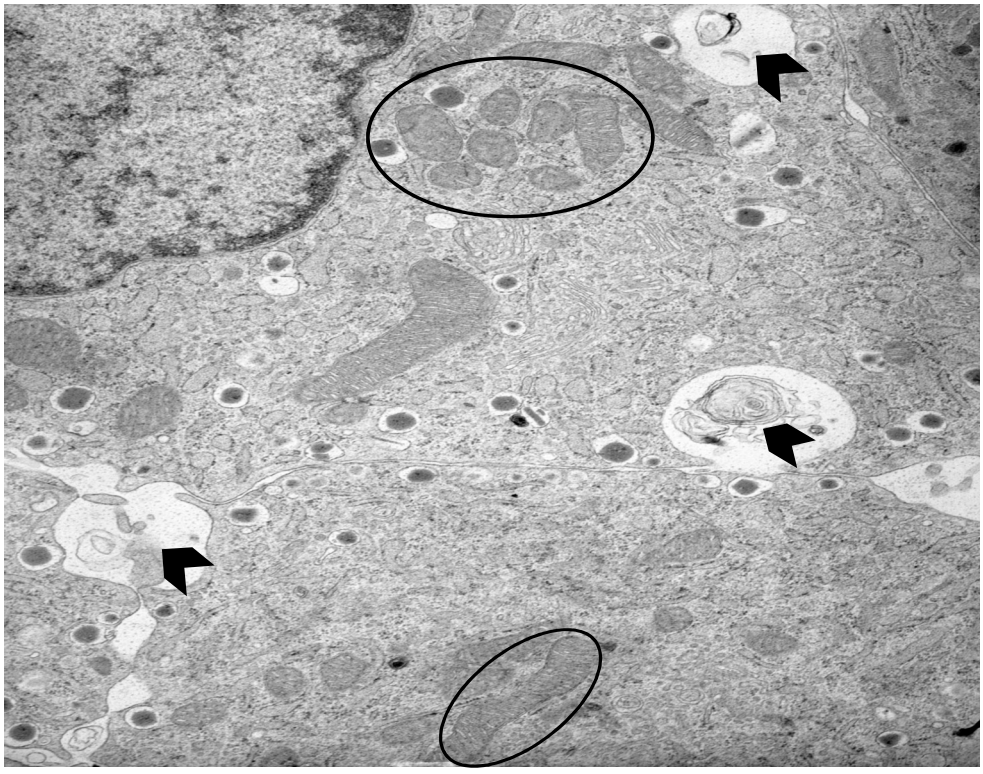


Figure 4.47 Ultrastructural changes produced by culturing NMRI islets in and RPMI containing 5mM glucose and gatifloxacin (10 or 100 µM) for 20h. **A)** Effect of 10 µM: Note the enlarged RER (arrow) and the β cell showing partial degranulation (asterisk) compared to the neighbouring β cells. **B)** Effect of 100 µM: Note the elongated mitochondrion (upper circle) and the mitochondria showing fusions (lower circle) in addition to the increased intercellular space (arrow head) that may correlate with degranulation observed. Magnification 8000 x



A



B

Figure 4.48 Ultrastructural changes produced by culturing NMRI islets in and RPMI containing 5mM glucose and ciprofloxacin (10 or 100 µM) for 20h. **A)** Effect of 10 µM: Note the elongated mitochondria (M1 and M2) and the mitochondrion showing fusion (M3).The arrow heads point to the increased intercellular space. **B)** Effect of 100 µM: Note the elongated mitochondrion (lower circle) and multiple mitochondria showing fusions (upper circle) in addition to the increased intercellular space (arrow head) that may correlate with degranulation observed. Degranulation is more widespread than with100 µM gatifloxacin. Magnification 8000 x

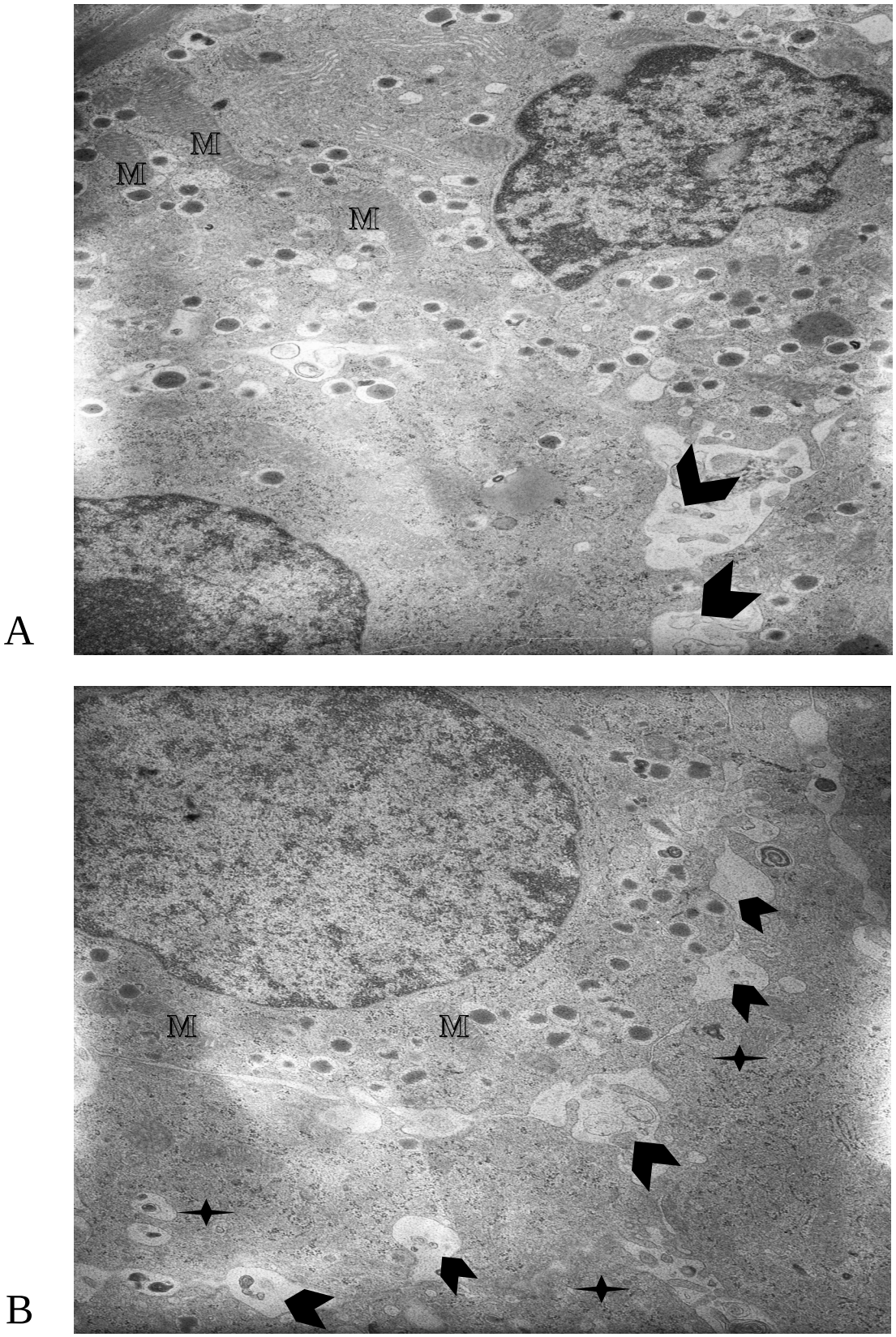


Figure 4.49 Ultrastructural changes produced by culturing NMRI islets in and RPMI containing 5mM glucose and moxifloxacin (10 or 100 µM) for 20h.A) Effect of 10 µM: The arrow heads point to the increased intercellular space and occasional elongated mitochondria (M). **B)** Effect of 100 µM: occasionally elongated mitochondrion and multiple mitochondria (M) showing fusions in addition to the increased intercellular space (arrow head) that may correlate with degranulation (asterisk) observed. Degranulation is more widespread than with100 µM moxifloxacin. Magnification 8000 x

5. Discussion

This study was carried out to explore the cytotoxic effects of fluoroquinolones and possible mechanism(s) that could explain their dysglycemic complication.

5.1 Digestion and culture of the pancreatic islets as well as β cells

The isolation of pancreatic islets using collagenase solution had begun with the pioneer work of Moskalewski (1965). Later on, Lacy and Kostianovsky (1967) introduced an islet isolation technique in rats that involved the ductal distension of the pancreas and collagenase digestion. Differences among various batches of collagenase including enzyme activity, purity, and formulation strongly influence the outcome of the islet isolation. Collagenase had been tested by Wolters et al. (1992) and de Haan et al. (2004) for proper digestion of rat islets. Bucher et al. (2004) also concluded that Serva collagenase was as effective as Liberase (Roche) in terms of islet yields and function. It might be argued that mouse islets are more vulnerable than rat islets to any potential deleterious effect of the collagenase during the isolation procedure. On the contrary, the integrity had been proved to be preserved by others (Zawalich et al., 2004).

Animal's age can influence the numbers of islets obtained. Although younger mice (less than 12 weeks) can give high yields of the islets, the injection into the small cholecystic duct was not practically feasible. Nevertheless, there are many factors that contribute to immaturity of β cells and diminished insulin secretory response to glucose and other secretagogues in neonatal cells compared with adult cells (Navarro-Tableros et al., 2007).

Studies of optimal culture conditions demonstrated that RPMI 1640 with serum maintains or augments glucose-stimulated insulin secretion in murine islets (Andersson, 1978). The role of serum for GSIS, insulin biosynthesis and islet cell survival had been confirmed (Ling and Pipeleers, 1994). Glucose concentration in the culture medium is at the centre of a delicate balance between necessary source of energy and islet toxicity. The choice of the glucose concentration of 5 mM was made so as not to stimulate the glucose metabolism during the cultivation period (Gilon et al., 1994) as extended exposure to high glucose may cause toxicity (Robertson et al., 2003). An increase in β-cell apoptosis has also been reported in rodent islets and insulin-secreting MIN6 cells cultured up to one week in the presence of a low non-stimulating glucose concentration (Efanova et al., 1998; Van de Casteele et al., 2003). However, in the current study, no negative effects were observed. This was exemplified by the response of the islets and/or β cells to potassium depolarization (calcium measurements) and response to 20 mM glucose when different parameters of mitochondrial energetics had been recorded. On the other hand, when the islets were incubated with 10 mM glucose, the functional integrity was compromised.

For each test series, pancreatic islets from two different preparations were used to eliminate the possibility of islet-to-islet communication that might be claimed to be responsible for the particular biological behaviour of islets (Giordano et al., 1993; Nunemaker et al., 2005).
The pancreatic islets with a small diameter were preferentially chosen as their functional integrity was often less compromised during their cultivation phase (Gilon et al., 1994) and the loading with the indicator was more homogeneous (Gilon et al., 1994; Nadal et al., 1999). Notably, large islets were more prone to central necrosis than small islets (Cui et al., 2005; Lehmann et al., 2007).
This "injection method" when compared to "conventional" digestion method had 3 advantages:

1. Free islets recovered from the pancreas were significantly more (150-200 islets / per pancreatic mouse) as perifusion through the pancreatic duct allowed collagenase to access the islets using anatomical structures.
2. The pancreatic islets during this isolation procedure were subjected to less mechanical damage (Carter et al., 2009).
3. The time required for attachment of the pancreatic islets to the cover slips was clearly diminished (12 -24 h) in comparison to that obtained using the "conventional" method (48-72 h).

Although Ca^{2+} signals can be registered with a higher temporal resolution in primary β cells, the pancreatic islets represent a good model to evaluate the effect of fluoroquinolones on $[Ca^{2+}]_i$ for the following reasons:

1. It allowed a direct comparison with results from experimental studies to determine the rate of insulin secretion. When dispersed, β cells exhibit multiple metabolic glucose-response populations with different insulin secretion properties (Bennett et al., 1996). In the intact pancreatic islets, intercellular signalling can occur via both chemical (paracrine; Rorsman et al., 1989; Salehi et al., 2005) and electrical signals (via gap junctions; Meda, 1996; Charollais et al., 2000). Both pathways of communication are disrupted when the islet is dissociated into single cells.

2. Studies of the intact islet reflect interactions between different cell types. The effects of non-dispersed β cells in an intact pancreatic islet are the more actual physiological conditions than individual β cells (Pipeleers et al., 1982; Jain and Lammert, 2009).

All experimental protocols were performed at a temperature of 35-36°C as cooling had been shown to inhibit not only the glucose-induced $[Ca^{2+}]_i$ rise but also Ca^{2+}-activated exocytosis (Niwa et al., 1996; Kinard and Satin , 1996).

5.2 Effects of fluoroquinolones on stimulus secretion coupling

5.2.1 Depolarizing effect

When exploring the mechanism of fluoroquinolone-induced insulin release, we first considered the K_{ATP} channel-dependent mechanism for glucose-stimulated insulin secretion (Maeda et al., 1996). It is generally accepted that a high degree of blockade of the K_{ATP} channel with a subsequent depolarization of the plasma membrane of β cells (in mice) stimulates insulin secretion even at very low glucose concentrations (Ashcroft and Ashcroft, 1990; Panten et al., 1992). It is generally believed that compounds with a direct blocking effect on K_{ATP} channels act as both initiators and enhancers of insulin secretion (Rustenbeck et al., 1997b; Bleck et al., 2004). However, it seems that the greater glucose dependence of fluoroquinolones effects on insulin secretion was not simply a function of K_{ATP} channel closure. The results obtained in the perforated patch mode (i.e. using metabolically intact β cells exposed to 5 mM glucose) resolved several of these contradictions (Ghaly et al., 2009). Using 100 μM of ciprofloxacin, gatifloxacin and moxifloxacin were virtually ineffective, corresponding to the lack of insulinotropic effect at 5 mM glucose. The loss of potency in the perforated patch mode (fig 5.1), as compared to the whole cell mode (fig 5.2), may be explained by the hypothesis that the inhibition of K_{ATP} channel activity by fluoroquinolones was susceptible to negative modulation by endogenous compounds, which were present in metabolically intact β cells, but not in open β cells (whole cell mode), where the cytosol was washed out by the pipette solution (Rustenbeck et al., 1997a; Strauss et al., 2001). In this context, the observation by Saraya et al. (2004) that gatifloxacin was more potent to block reconstituted Kir 6.2/ SUR1 channels in HEK cells (Human Embryonic Kidney) than to block native K_{ATP} channels in MIN6 cells can be seen as indirect evidence for the existence of negative modulators in insulin-secreting cells.

5.2.2 Effects of fluoroquinolones on $[Ca^{2+}]_i$

5.2.2.1 Effects on $[Ca^{2+}]_i$ in NMRI mice

Numerous experiments conducted over many years have established that insulin secretion is calcium dependent (Satin, 2000) as a result of blockade of K_{ATP} channels (Valdeolmillos et al., 1992). As depolarization leads to the opening of VDCC channels producing an increase in $[Ca^{2+}]_i$, which stimulates insulin granule exocytosis (Renström et al., 1996), so the next step was to measure $[Ca^{2+}]_i$. Fura-2 is currently very popular Ca^{2+} indicator. The larger fluorophore of Fura-2 (compared to quin-2) gives it slightly longer wavelengths of excitation compatible with

glass microscope optics, a larger extinction coefficient and a higher quantum efficiency (Tsien, 1989).

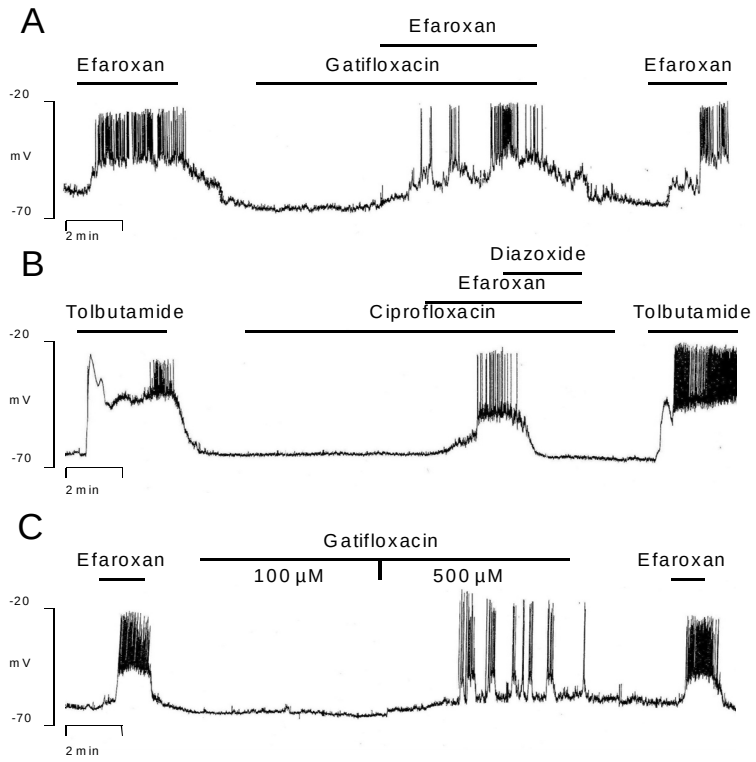


Figure 5.1 Comparison of the depolarizing effect of fluoroquinolones on metabolically intact pancreatic β -cells. Original registrations of the effect of 100 µM of gatifloxacin (A), ciprofloxacin (B) and 500 µM gatifloxacin (C) on the membrane potential of mouse pancreatic β cells in the perforated patch configuration. At 100 µM none of the fluoroquinolones elicited a depolarization. Note the effect of 100 µM of efaroxan in the presence of 100 µM gatifloxacin or ciprofloxacin (A and B). Increasing gatifloxacin concentration from 100 to 500 µM led to action potential spiking interrupted by short phases of repolarisation (C). Typical registrations were made of 3–4 experiments.

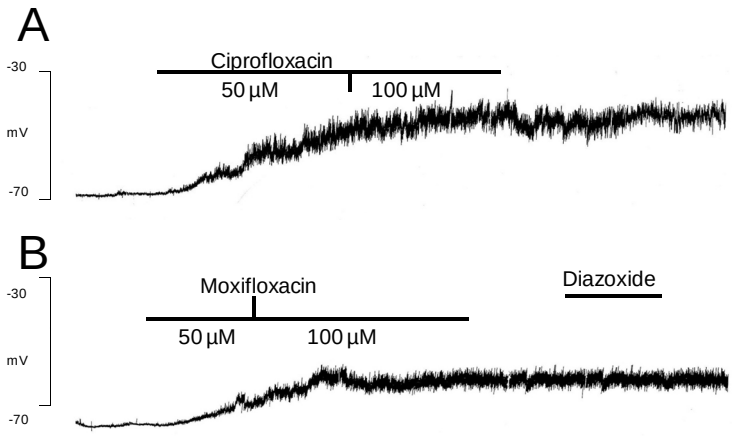


Figure 5.2 Depolarization of the membrane potential of mouse pancreatic β cells by ciprofloxacin (A), moxifloxacin (B). Original registrations of the membrane potential of cultured mouse β cells were done using the conventional whole cell-configuration. Initially, the fluoroquinolone concentration was 50 µM, which was increased to 100 µM when a steady state was apparently reached. Typical registrations were made of 4–7 experiments.

The microfluorometric measurements of fluoroquinolone induced $[Ca^{2+}]_i$ changes in β cells was difficult to be assessed because the endogenous fluorescence of the fluoroquinolones (Idowu and Peggins, 2004) interfered with the Fura-fluorescence. The use of D600 confirmed that the $[Ca^{2+}]_i$ increase was due to depolarization-induced influx of Ca^{2+} via L-type Ca^{2+}channels (Dryselius et al., 1999).

At non-stimulatory glucose concentration, neither of the 3 drugs had proved to affect $[Ca^{2+}]_i$ except when the concentration was increased from 100 to 500 μM, which was far above the maximal serum level in healthy subjects during therapeutic dosages. Only 500 μM ciprofloxacin produced an increase $[Ca^{2+}]_i$ that was irreversible even by the use of D600 or calcium free perifusion medium, where tolbutamide effect disappeared, indicating that release from internal stores may play an additional role (Loo et al., 1997).

In the presence of 10 mM glucose, both gatifloxacin and moxifloxacin induced a strong increase of $[Ca^{2+}]_i$ whereas ciprofloxacin was moderately effective. The marked $[Ca^{2+}]_i$ increase by gatifloxacin fits to the secretory characteristics. This had been successfully, though incompletely, counteracted by K^+ channel opening using diazoxide while the remaining effect was further reduced by the calcium channel blocker D600. The moderate but continuing $[Ca^{2+}]_i$ increase by ciprofloxacin and the magnitude of the moxifloxacin-induced increase of $[Ca^{2+}]_i$ were unexpected. These observations concur with the marked enhancing effect of ciprofloxacin and moxifloxacin on secretion when the glucose concentration was raised to a stimulatory level. Unlike sulfonylureas, it seems that fluoroquinolones are not stimulatory for insulin secretion but rather enhance the glucose induced secretion (Ashcroft, 2000). Rather, this mode of action resembles that of imidazolines (Rustenbeck et al., 1997b; Proks and Ashcroft, 1997). They enhance the effect of a stimulatory concentration of glucose-induced insulin secretion by activation of K_{ATP} channel via the pore-forming Kir6.2 subunit (Rustenbeck, 1999; Grosse-Lackmann et al., 2003). The presence of SUR1 either increased the affinity of the binding site, or enhanced the efficacy with which drug binding is converted into channel closure (Zünkler et al., 2006).

To confirm the functional integrity of the cultured pancreatic islets/ β cells, they were exposed to a maximum of depolarizing K^+ concentration. This stimulus, regardless of the glucose concentration, evoked an increase in the effective cytosolic Ca^{2+} concentration (Gilon and Henquin, 1992; Rustenbeck, 1999).

5.2.2.2 Effects on $[Ca^{2+}]_i$ in SUR1 knockout mice

The SUR1 knockout mouse model was used to study possible effects of fluoroquinolones on depolarization independent-Ca^{2+} transport. SUR1 knockout mice were used instead of the pharmacological inhibitors with which non-specific effects can never be entirely excluded. Although this kind of K_{ATP}-channel-deficient mice were used to test the amplifying pathway, but signals of a triggering pathway were supposed to exist (Haspel et al., 2005). This transgenic animal model was developed in a working group by Lydia Aguilar-Bryan and Joseph Bryan (Seghers et al., 2000). As both Kir6.2 and SUR1 subunits are normally required for functional expression of the K_{ATP} channel, the inactivation of SUR1 part based on the mutation of the ABCC8 coding gene prevented the constitution functional channel (Inagaki et al., 1995b; Zerangue et al., 1999). The SUR1 gene is located within a region on chromosome 11 and was reported to be linked to hyperinsulinemia of infancy (Thomas et al., 1995).

The physiological and functional properties of both SUR1KO pancreatic islets as well as the β cells were characterized in some working groups (Seghers et al., 2000; Nenquin et al., 2004; Marhfour et al., 2009). The absence of functional K_{ATP} channels was associated with a partial depolarization of the plasma membrane, with continuous Ca^{2+} action potentials and increased $[Ca^{2+}]_i$ values independently from extracellular glucose concentration (Düfer et al., 2004; Szollosi et al., 2007). The ability of isolated SUR1KO islets to respond to a maximum K^+ concentration, irrespective of the K_{ATP} channel, was used as an internal standard (Szollosi et al., 2007)

Gatifloxacin was the most efficient to increase $[Ca^{2+}]_i$ followed by ciprofloxacin and then lastly moxifloxacin. The partial antagonism brought about by D600 with gatifloxacin and ciprofloxacin and modest one seen with moxifloxacin, still implicating Ca^{2+} influx via L-type Ca^{2+} channels. However, knockout β cells have a chronically elevated $[Ca^{2+}]_i$ because the smaller K^+ conductance leads to a partial depolarization. It appears that this increase of $[Ca^{2+}]_i$ is not related to stimulated insulin secretion since fluoroquinolones did not enhance insulin secretion from SUR1KO islets (Ghaly et al., 2009), also moxifloxacin did not produce dysglycemia in diabetic patients (Barth and Landen, 2003; Lipsky et al., 2007; Vick-Fragoso et al., 2009). Dissociations between $[Ca^{2+}]_i$ increases and stimulation of secretion have been found earlier (Zaitsev et al., 1995).

The moderate $[Ca^{2+}]_i$ increase by fluoroquinolones in islet cells from SUR1KO mice, which do not have functional K_{ATP} channels, suggests that fluoroquinolones may have additional sites of action by which $[Ca^{2+}]_i$ is affected. Cytosolic calcium rises are relayed to mitochondria, where the signal is amplified (Kennedy et al., 1996). While calcium influx across the plasma

membrane is the primary signal for insulin granule exocytosis, the recruitment of granules depends on cellular energy metabolism (Varadi et al., 2002). Calcium has been proposed to modulate mitochondrial function with a direct impact on intermediary metabolism, substrate oxidation and energy production.

5.2.3 Effect on reducing equivalents

As fluoroquinolones (100 µM) depolarized open cell preparations of β cells, but none of these compounds depolarized metabolically intact β cells which was only seen using 500 µM, so an interference with energy metabolism was supposed to play a role. Certain parameters of the mitochondrial energetics had been examined; namely NAD(P)H, FAD, $\Delta\Psi m$, ATP/ADP ratio.
Therefore, fluorescence measures of NAD(P)H and FAD can be used as a measure of intracellular redox state (Chance et al., 1979). Flavoproteins signals have been used much less than NAD(P)H signals in studies of β cell energetics. However flavoprotein autofluorescence had also been demonstrated to have significant utility for studies of mitochondrial energetics in β cells and HIT cell line (Panten and Ishida, 1975; Duchen, et al., 1993; Civelek et al., 1996). The signals generated were assumed to be due to mitochondrial dynamics. Early work concluded that multiple flavoproteins were involved in mitochondrial electron transfer and were thereby coupled to NADH utilization by the electron transport chain (Scholz et al., 1969). Many studies demonstrated that flavoprotein metabolic signals could be more direct measures of oxidative metabolism and less contaminated by cytosolic processes than NAD(P)H measurements (Shuttleworth, 2010).
The used protocol to elicit recognizable changes was to increase glucose concentration to 20 mM which increased both β cell FAD and NAD(P)H fluorescence with minimal contribution from non β cell (Quesada et al., 2006; Smelt et al., 2008).
In contrast to the heterogeneous responses produced in isolated β cells, glucose induced a uniform increase in NAD(P)H autofluorescence in β cells residing within intact islets (Bennett et al., 1996). Experiments where exposure to the fluoroquinolones took place in the presence of glucose showed contribution of fluoroquinolones much higher than that of NAD(P)H. Increased cellular accumulation may be due to increased uptake into the mitochondria. However, indirect effect by ATP-dependent processes is also possible. Cellular accumulation of fluoroquinolones was also reported with gatifloxacin using the primary cultured rat brain micro-vessel endothelial cells (rBMECs) as an in vitro model for transport across blood brain barrier (Li et al., 2009). For moxifloxacin, the use of sodium fluoride, azide, and cyanide interfered with the transport of the drug into the phagosomes (Hall et al., 2003) and similar observations were made in human polymorphonuclear leucocytes (Pascual et al., 1999). Ciprofloxacin accumulation in

polymorphonuclear leucocytes had been attributed to protein kinase C up-regulation (Walters and Nakkula, 2003).

When the islets were pre-incubated with the drugs for one hour, the increase of NAD(P)H autofluorescence, that was typically elicited by 20 mM glucose, was abolished by moxifloxacin treatment but only moderately reduced by ciprofloxacin or gatifloxacin. The same effect was observed using islets of SUR1KO mice indicating that this inhibitory effect was independent on the blocking effect that fluoroquinolones exerted on the K_{ATP} channels considering that the glucose metabolism in SUR1KO islets was not altered. The NAD(P)H results of control experiments of SUR1KO islets reflected the unimpaired oxidation of glucose which is in accordance with Szollosi et al. (2007).

Although the three fluoroquinolones increased $[Ca^{2+}]_i$ with variable magnitude, but the net effect of $[Ca^{2+}]_i$ on NAD(P)H autofluorescence is dependent upon the degree of respiratory chain flux, and may represent a balance of activation of calcium sensitive mitochondrial dehydrogenases on one hand, and Ca^{2+}-induced NAD(P)H oxidation on the other (Civelek et al., 1996).

Concerning FAD, moxifloxacin and, to a lesser degree, ciprofloxacin attenuated the glucose-induced decrease of autofluorescence which was a more specific indicator of the intra-mitochondrial production of reducing equivalents (Husson et al., 2007). On the other hand, gatifloxacin produced an effect close to the control experiment.

5.2.4 Mitochondrial membrane potential

Central to mitochondrial function is an electrochemical proton gradient across the mitochondrial inner membrane that is established by the proton pumping activity of the respiratory chain. The proton gradient establishes a proton-motive force, which has two components: a pH differential and an electrical membrane potential ($\Delta\Psi m$). The pH component of mitochondrial proton motive force is small relative to the membrane potential and, hence, it is the latter that provides the predominant driving force (Campanella et al., 2009).

It seems that moxifloxacin, and to lesser extent gatifloxacin, causes hyperpolarizing effect in the presence of non-stimulatory glucose level. Interestingly, only moxifloxacin somewhat reduced the effect of uncoupler CCCP even when wash out while the effect of azide is still preserved suggesting that moxifloxacin may have some effect on the respiratory chain. Ciprofloxacin had neither effect on the mitochondrial membrane potential nor on the uncoupler.

Ideally, the effect of fluoroquinolones on mitochondrial membrane potential was to be demonstrated with the same experimental conditions under which the effect on the reducing equivalents was measured. However, when the pre-incubation protocol was used (which was not necessary since the fluorescence excitation of Rh123 and that of fluoroquinolones did not

overlap) there was no effect of the fluoroquinolones. This surprising lack of effect may be explained by the hyperpolarizing effect of the fluoroquinolones in the presence of 5 mM glucose (above). Thus, it can be assumed that a possible reduction of the glucose induced hyperpolarization was offset by the direct hyperpolarizing effect of the fluoroquinolones themselves. This assumption proved to be correct when the fluoroquinolones were added after 20 mM glucose had led to hyperpolarization. Again moxifloxacin was the most efficient compound to diminish the glucose induced hyperpolarization.

This may be explained by that the islet mitochondria appeared to be more energized in comparison to the mitochondria from dispersed β cells. The differences in mitochondrial energy storage may be due, in part, to the higher levels of $[Ca^{2+}]_i$ in dispersed β cells, which, in turn, may depolarize ($\Delta\Psi m$) to a greater extent. The elevated calcium levels observed in dispersed β cells could have an effect on communication between the mitochondria and the cell membrane. The incorporation of β cells into islet thus may help them to maintain a larger mitochondrial gradient for increased ATP production (Nunemaker and Satin, 2004).

5.2.5 Effect on ATP/ADP ratio

Since the data so far suggested that the inhibitory effect of fluoroquinolones on the mitochondrial energy metabolism, it was of interest to measure the islet content of ATP and ADP as well as ATP/ADP ratio.

When the pre-incubation protocol was performed, neither gatifloxacin nor ciprofloxacin significantly affected the ATP/ADP ratio. Only moxifloxacin significantly reduced ATP/ADP ratio which is confirmatory to its inhibitory effect on mitochondrial energetics. This effect was not observed using basal glucose concentration which corresponds to the absence of depolarizing effect of moxifloxacin in the perforated patch mode.

While a correspondence could be found between the marked effect of moxifloxacin on the NAD(P)H fluorescence and the effect on adenine nucleotide content of pancreatic islets, no such relation was apparent when the direct effect of fluoroquinolones was tested in the presence of 5 mM glucose. This experimental protocol had been chosen because measurements of the mitochondrial membrane potential using the indicator Rh123 had shown that the fluoroquinolones exert a hyperpolarizing effect. Theoretically, this could be due to an increased offer of reducing equivalents to the respiratory chain (this causes the hyperpolarization by 20 mM glucose) (Duchen et al., 1993), however, the more likely explanation would be that the respiratory chain was inhibited by the fluoroquinolones at a distal site with an unchanged offer of reducing equivalents from the Krebs cycle (Rustenbeck et al., 1997a). Apparently, this inhibition if it takes place cannot reach the threshold to reduce the oxidative phosphorylation

(Gallagher et al., 1986; Rossignol et al., 2003). An alternative explanation could be that the fluoroquinolones somewhat reduce the proton leak across the inner mitochondrial membrane. This would fit to the observation that moxifloxacin apparently counteracted the depolarization by CCCP. On the other hand, this reduced depolarization did not translate into a diminished effect of CCCP on the adenine nucleotide content.

While the adenine nucleotide measurements by themselves contain logic in that the offer of glucose or the uncoupling of the respiratory chain lead to the expected effects, the effect of the fluoroquinolones is less straightforward to interpret than expected. There is scarce literature dealing with the effects of fluoroquinolones on β cell mitochondria. Meanwhile the inhibitory effect of some fluoroquinolones on mitochondrial functions was observed in primary human osteoblasts and tendon cells (Duewelhenke et al., 2007; Lowes et al., 2009). To further clarify the mechanism of action at the mitochondria will require the measurement of an additional parameter, namely the oxygen consumption. Only by this way, it will be possible to determine whether the changes in membrane potential are due to inhibition of the respiratory chain enzymes or to changes in the proton leak size.

It is not surprising that quinolones can compromise the mitochondrial energetics as antibacterial agents are commonly known to impair mitochondrial function, some acting as inhibitors of mitochondrial RNA and protein synthesis (Zhang et al., 2005), while others acting as inhibitors or uncouplers of oxidative phosphorylation (Gallagher et al., 1986).

5.2.6 Ultrastructural changes induced by fluoroquinolones

In the functional experiments the exposure to the fluoroquinolones was much shorter than during an antibiotic therapy, i.e. 1 h vs 72 h (most of the fluoroquinolone-induced hypoglycaemias occur during the first 3 days). As so often in pharmacology the compressed time scale was compensated by increased concentration. 100 µM was the concentration which regularly produced an increase in insulin secretion when the glucose concentration was stimulatory (10 mM). This is why this concentration was chosen for the experiments to characterize the mitochondrial effects. On the other hand, it is clearly above the peak plasma concentrations even though not completely beyond measure. For this reason the effect of 10 µM and 100 µM of the three selected fluoroquinolones on beta cell ultrastructure was investigated. The electromicrographs confirmed that fluoroquinolones enhance the insulin secretion, since at 100 µM a degranulation was clearly visible. The nearly unchanged content of granules at 10 µM is probably due to the non-stimulatory glucose concentration (5 mM) during the cell culture period.

While this observation is similar to that made with sulfonylurea-exposed islets (with the difference that sulfonylureas induce a stronger degranulation, sometimes even a complete emptying of the granule pool, Rustenbeck et al., 2004), the changes in mitochondrial morphology appear to be more specific for this group of compounds. Mitochondrial EM changes after sulfonylurea exposure showed enhanced content of mitochondria and increased mitochondrial volume (Borg and Andersson, 1981). First generation imidazolines, which similar to fluoroquinolones block K_{ATP} channels by binding to the pore-forming subunit (Grosse-Lackmann et al., 2003), did not produce such changes. On the other hand, it ought to be mentioned that beta cells from K_{ATP} of SUR1KO mice contain increased numbers of mitochondria, many of which are elongated or otherwise enlarged (Jörns and Rustenbeck, unpublished). So it cannot be ruled out that there may be a causal relation between the ability to depolarize the beta cell plasma membrane and the induction of changes in mitochondrial morphology. On the other hand, the functional changes induced by all three fluoroquinolones in SUR1KO islets suggest that the mitochondrial effects are not dependent on the existence of a K_{ATP} channels in the plasma membrane (see chapter 4.2.3.2). This view concurs with published observation of mitochondrial effects of fluoroquinolones in other tissues e.g. skin fibroblasts and chondrocytes (Ouédraogo et al., 2000; Stahlmann et al., 2000; Kato, 2008). So the hypothesis needs to be tested that the changes in beta cell mitochondrial morphology are due to direct effect on the mitochondrial metabolism.

5.3 Correlation of the cytotoxic effects with dysglycemic episodes

Among the currently used fluoroquinolones, gatifloxacin appears to have consumed much of the current focus (Frothingham, 2005). Compared to other broad-spectrum oral antibiotics, gatifloxacin causes a greater risk of hospitalization for treatment of dysglycemia, and is associated with nearly a 17-fold higher risk of hyperglycemia (Park-Wyllie et al., 2006). Moreover, it is distinguished from other fluoroquinolones by its exceptional ability to produce hypoglycemia and hyperglycemia (Khovidhunkit and Sunthornyothin, 2004; Haerian et al., 2008).

Several lines of evidence show that the drug concentrations used in the present work correspond to values achieved after administrations of therapeutic doses. Regarding gatifloxacin:

1. A large volume of distribution of gatifloxacin in the pancreas had been shown to play a pivotal role in the high occurrence of dysglycemia (Nagai et al., 2010). It is well distributed in tissues, often achieving levels that exceed its plasma concentrations (Grasela, 2000).

2. Past investigations on the mechanism of fluoroquinolone-induced dysglycemia usually adopted that the blood concentration of gatifloxacin in patients with glycemic abnormality episodes has been ranged from 4.21 to 5.80 μg /ml (from 10.5 to 14.4 μM) while its concentration in pancreatic tissue 1 h after single oral administration was about six times higher than blood concentration in rats. The pancreatic gatifloxacin concentration (128 μg/g) was higher than that of lomefloxacin (69 μg/g) or levofloxacin (39 μg/g), although there was little difference in serum drug concentrations (12 to 15 μg/ml) among them (Saraya et al., 2004; Ishiwata et al., 2006a and 2006b; Yamada et al., 2006; Tomita et al., 2007; Furuhama, 2007).
3. In patients with renal impairments, plasma gatifloxacin levels may double the values mostly detected in healthy subjects (Ishiwata et al., 2006b). Moreover, the mean elimination half-life of gatifloxacin is relatively long (about 7 h) and urinary excretion of unchanged drug is the major route of elimination (Fish and North, 2001).

Similarly, simultaneous measurements of pancreatic tissue and plasma concentrations have demonstrated that moxifloxacin readily penetrates into human pancreatic tissue following intravenous or oral administration (Wacke et al., 2006). In case of ciprofloxacin, tissue levels exceeding the respective serum levels have been reported from human pancreas (Isenmann et al., 1994).

None of the fluoroquinolones stimulated insulin secretion in the presence of a basal glucose concentration (5 mM), but raising the glucose concentration from 5 to 10 mM revealed the insulinotropic effect. Thus, the insulinotropic effect of the fluoroquinolones is not an initiation of insulin secretion, but rather an enhancement of the stimulatory effect of β cell nutrients.

We assume that the opposing effects produced by moxifloxacin (on $[Ca^{2+}]_i$ and mitochondrial de-energization) could explain why this drug is the least to influence glucose homeostasis.

Several approaches using intact or permeabilised cells have shown the involvement of ATP at steps of the secretory process distal to the $[Ca^{2+}]_i$ increase. So, it is not surprising that increased $[Ca^{2+}]_i$,when associated with a decrease in the energy state of islets/ β cells, cannot augment insulin release (Detimary et al., 1994).

The results described herein are consistent with clinical experience that among the fluoroquinolones, gatifloxacin is associated with the highest risk of hypoglycemia. On the other hand, large analyses of pooled data from patients receiving moxifloxacin fail to identify any glucose derangement, which could be partially attributed to the pharmacokinetic properties of the drug being not renally excreted (Gavin III et al., 2004).

6. Summary and Perspective

Fluoroquinolones are nowadays an important class of antibacterial agents, possessing a broad spectrum of activity against Gram-positive, Gram-negative, and mycobacterial organisms, as well as against anaerobes including otherwise resistant strains. In particular, treatment of tuberculosis by fluoroquinolones is an area of growing importance. Ciprofloxacin and moxifloxacin were shown to exert antineoplastic activity in several cancer cell lines. Although they are generally well tolerated, newer quinolone derivatives have caused several unexpected adverse reactions including severe dysglycemic episodes. Several agents had been withdrawn from the market for this reason, such as temafloxacin, clinafloxacin and gatifloxacin. Given the frequency with which this group is prescribed and that dysglycemia may be not correlated to the intake of these drugs especially in diabetics, cases may be underreported in published reports which are strictly conducted in hospitalized patients. The true incidence could only be determined through prospective, population-based surveillance.
Three clinically relevant fluoroquinolones were selected for this study: gatifloxacin, ciprofloxacin and moxifloxacin. Gatifloxacin is the fluoroquinolone which is the most associated with disturbances of the glucose homeostasis, including several fatalities. Therefore, its use is practically discontinued, even though it is a highly effective antibacterial agent. Ciprofloxacin can be regarded as the standard fluoroquinolone, there is a number of case reports of serious hypoglycemias associated with its use, but the overall risk of dysglycemias is generally regarded as acceptable. Moxifloxacin, even though structurally closely similar to gatifloxacin, is not associated with serious hypoglycemias.

Originally, it was believed that fluoroquinolones would only aggravate sulfonylurea-induced hypoglycemias, but it is meanwhile clear that co-administration with sulfonylureas is an independent risk factor and that fluoroquinolones are inducers of hypoglycemia in their own right. In fact, fluoroquinolones increase insulin secretion by inhibiting the activity of the K_{ATP} channels of the pancreatic β cell. However, investigations preceding this thesis work have come to the conclusion that the insulinotropic effect is not sufficiently explained solely by the ability of the fluoroquinolones to block K_{ATP} channels in pancreatic β cells.

This conclusion was based on a number of observations. In particular, the observation that fluoroquinolones were able to depolarize open cell preparation of pancreatic beta cells and thus to initiate the well-known sequence of opening of voltage-dependent Ca^{2+} channels and triggering the Ca^{2+} dependent exocytosis, but were unable at an equimolar concentration to do the same with metabolically intact β cells (perforated patch configuration) suggested that a

metabolism-dependent endogenous factor might counteract the K_{ATP} channel-blocking effect of the fluoroquinolones.

Supposing that an interference with mitochondrial energy metabolism was the reason for this discrepancy, the effect of gatifloxacin, ciprofloxacin and moxifloxacin on NAD(P)H- and FAD-autofluorescence, the mitochondrial membrane potential (Rhodamine 123-fluorescence) and adenine nucleotide content of isolated pancreatic islets were investigated in this thesis. Initially however, the $[Ca^{2+}]_i$ concentration was measured to check whether the depolarizing ability (or inability) of the fluoroquinolones translated directly into changes of the cytosolic Ca^{2+} concentration which is regarded as the direct triggering factor of exocytosis.

In fact, it could be shown that in the presence of a basal glucose concentration (5 mM) all three fluoroquinolones were unable to increase $[Ca^{2+}]_i$ at 100 µM while at 500 µM a clear increase of $[Ca^{2+}]_i$ could be observed. This concentration dependency concurs with that to depolarize metabolically intact β cells. In the presence of 10 mM glucose, which by itself raises $[Ca^{2+}]_i$, a clear further increase of $[Ca^{2+}]_i$ was produced by all compounds, which was partially antagonized by D-600, a blocker of L-type Ca^{2+} channels and by diazoxide, an opener of K_{ATP} channels. Thus it can be concluded that a depolarization-induced Ca^{2+} influx plays a role in the $[Ca^{2+}]_i$ increase, but does not wholly explain it. This hypothesis was confirmed by the observation that all three fluoroquinolones at 100 µM produced a moderate but unmistakable $[Ca^{2+}]_i$ increase in SUR1KO islets, which do not have functional K_{ATP} channels. Apparently, fluoroquinolones affect $[Ca^{2+}]_i$ by one or more mechanisms unrelated to the block of K_{ATP} channels.

Since the fluoroquinolone fluorescence interfered with the measurement of the NAD(P)H- and FAD-autofluorescence of the perifused islets, the exposure to the fluoroquinolones took place during a 1 h preincubation period, followed by a perifusion first without glucose and then with glucose at a stimulatory concentration. 100 µM of moxifloxacin, but not of ciprofloxacin or gatifloxacin abolished the NAD(P)H increase typically elicited by 20 mM glucose. Similarly, moxifloxacin and, to a lesser degree, ciprofloxacin diminished the glucose-induced decrease of FAD-fluorescence, which is a more specific indicator of the intramitochondrial production of reducing equivalents. Moxifloxacin, but not ciprofloxacin or gatifloxacin significantly reduced the ATP-to-ADP ratio produced by 20 mM glucose, thereby confirming the inhibitory effect on mitochondrial energetics and at least partly explaining the lack of depolarizing effect in intact beta cells at basal glucose. However, in the presence of basal glucose (5 mM) a significant inhibitory effect on the ATP-to-ADP ratio could not be verified.

Initially, the effect of the fluoroquinolones on the mitochondrial membrane potential was measured using the same experimental protocol as for the measurement of the NAD(P)H- or FAD-fluorescence, i.e. after a preincubation period. Using this protocol, there was no obvious reduction of the glucose (20 mM) -induced hyperpolarization. However, when the exposure to the fluoroquinolones was done during the measurements, it was found that all three fluoroquinolones produced a slowly progressing hyperpolarization of the mitochondrial membrane potential. This effect was most marked with moxifloxacin, which even somewhat inhibited the depolarizing effect of the uncoupler, CCCP. When the fluoroquinolones were added after a stimulatory glucose concentration (20 mM) had established a hyperpolarized membrane potential of the mitochondria, an antagonizing effect of moxifloxacin, but not of gati- or ciprofloxacin became visible.

From the data obtained during this thesis work it can be concluded that fluoroquinolones affect β cells functions beyond blocking K_{ATP} channels. This is obvious in the case of the $[Ca^{2+}]_i$ increase in SUR1KO islets. This K_{ATP} channel-independent increase in $[Ca^{2+}]_i$ could be secondary to the interference of the fluoroquinolones with mitochondrial functions. Obviously, fluoroquinolones counteract the effect a stimulatory glucose concentration, but have little or no effect in the presence of a basal glucose concentration. Intriguingly, the most marked effect on the parameters of mitochondrial energetics is exerted by moxifloxacin. Given the central role of β cell energetics in the stimulus-secretion coupling, this effect of moxifloxacin may counteract the consequences of the K_{ATP} channel block for the release of insulin. This would explain why in clinical use this compound is the least associated with hypoglycemias.

Certainly, this hypothesis requires verification and a further elucidation of the mitochondrial site of action of the fluoroquinolones. The autofluorescence of the fluoroquinolones is a considerable obstacle in this regard, precluding a number of useful experimental protocols. To pinpoint the exact nature of this interference will thus require the measurement of additional parameters such as oxygen consumption. Finally, it cannot be excluded that other mechanisms not considered here may also affect insulin secretion such as Mg^{2+} chelation, suppression of GLUT1 function or interaction with the neurotransmitter, GABA. Because there is a growing need for antibacterial drugs, e.g. for the treatment of tuberculosis, and because the development of fluoroquinolones is ongoing, the knowledge which structural features contribute to the insulinotropic property may be decisive for the availability of effective and safe compounds of this class.

7. References

Aberer W, Bircher A, Romano A. Blanca M, Campi P, Fernandez J, Brockow K, Pichler WJ, Demoly P (2003): Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy; 58:854-863.

Adeghate E, Ponery AS (2003): Pancreatic peptides, neuropeptides and neurotransmitters in diabetes mellitus: a review. Int J Diabetes and Metabolism; 11:1-6.

Agbaht K, Bitik B, Piskinpasa S, Bayraktar M, Topeli A (2009): Ciprofloxacin-associated seizures in a patient with underlying thyrotoxicosis: Case report and literature review. Int J Clin Pharmacol Ther; 47 (5):303-310.

Ahmed AS, Khan NZ, Saha SK, Chowdhury MA, Muslima H, Law P, Islam M, Bhattacharya M, Darmstadt GL (2006): Ciprofloxacin treatment in preterm neonates in Bangladesh: Lack of effects on growth and development. Pediatr Infect Dis J; 25 (12):1137-1141.

Ahrén B (2000): Autonomic regulation of islet hormone secretion – implications for health and disease. Diabetologia; 43:393–410.

Akahane K, Kato M, Takayama S (1993): Involvement of inhibitory and excitatory neurotransmitters in levofloxacin- and ciprofloxacin-induced convulsions in mice. Antimicrob Agents Chemother; 37:1764–1770.

Akahane K, Sekiguchi M, Une T, Osada Y (1989): Structure-epileptogenicity relationship of quinolones with special reference to their interaction with gamma-aminobutyric acid receptor sites. Antimicrob Agents Chemother; 33(10):1704-1708.

Almers W, Neher E (1985): The Ca signal from fura-2 loaded mast cells depends strongly on the method of dye-loading. FEBS Lett; 192:13-18.

Ambrose PG, Bhavnani SM, Cirincione BB, Piedmonte M, Grasela TH (2003): Gatifloxacin and the elderly: pharmacokinetic-pharmacodynamic rationale for a potential age-related dose reduction. J Antimicrob Chemother; 52(3):435-440.

Anderson ME, Mazur A, Yang T, Roden DM (2001): Potassium current antagonist properties and proarrhythmic consequences of quinolone antibiotics. J Pharmacol Exp Ther; 296 (3):806-810.

Andersson A (1978): Isolated mouse pancreatic islets in culture: effects of serum and different culture media on the insulin production of the islets. Diabetologia; 14(6):397-404

Anello M, Lupi R, Spampinato D, Piro S, Masini M, Boggi U, Del Prato S, Rabuazzo AM, Purrello F, Marchetti P (2005): Functional and morphological alterations of mitochondria in pancreatic β cells from type 2 diabetic patients. Diabetologia; 48(2):282-289.

Aranda A, Mayorga C, Ariza A, Doña I, Rosado A, Blanca-Lopez N, Andreu I and Torres MJ (2010): In vitro evaluation of IgE-mediated hypersensitivity reactions to quinolones. Allergy, doi: 10.1111/j.1398-9995.2010.02460.x

Arce CA, Bhasin SRS, Pasmantier RM (2004): Severe hyperglycemia during gatifloxacin therapy in patients without diabetes. Endocr Pract; 10:40-44.

Ashcroft FM, Proks P, Smith PA, Ammala C, Bokvist K, Rorsman P (1994): Stimulus-secretion coupling in pancreatic β cells. J Cell Biochem; 55:54-65.

Ashcroft SJ (2000): The β cell K (ATP) channel. J Membr Biol; 176(3):187-206.

Ashcroft SJ, Ashcroft FM (1990): Properties and functions of ATP-sensitive K-channels. Cell Signal; 2(3):197-214.

Aspinall SL, Good CB, Jiang R, McCarren M, Dong D, Cunningham FE (2009): Severe dysglycemia with the fluoroquinolones: a class effect? CID; 49(3):402-408.

Azar S, Ramjiani A, Van Gerpen JA (2005): Ciprofloxacin-induced chorea. Movement Disorders; 20 (4):513-514.

Badshah A, Janjua M, Younas F, Halabi AR, Cotant JF (2009): Moxifloxacin-induced QT prolongation and torsades: An uncommon effect of a common drug. Am J Med Sci; 338 (2):164-166.

Baker SE, Hangii MC (2002): Possible gatifloxacin-induced hypoglycaemia. Ann Pharmacother; 36(11):1722-1726.

Ball P, Mandell L, Patou G, Dankner W, Tillotson G (2004): A new respiratory fluoroquinolone, oral gemifloxacin: a safety profile in context. Int J Antimicrob Agents; 23(5):421-429.

Ball P, Mandell L, Niki Y, and Tillotson G (1999): Comparative tolerability of the newer fluoroquinolone antibacterials. Drug Safety; 21:407-421.

Ballian N, Brunicardi FC (2007): Islet vasculature as a regulator of endocrine pancreas function. World J Surg; 31:705-714.

Bar-Oz B, Moretti ME, Boskovic R, O'Brien L, Koren G (2009): The safety of quinolones-a meta-analysis of pregnancy outcomes. Eur J Obstet Gynecol Reprod Biol; 143(2):75-78.

Barth J, Landen H (2003): Efficacy and tolerability of moxifloxacin in 2338 patients with acute exacerbation of chronic bronchitis. Clin Drug Invest; 23(1):1-10.

Basaria S, Cooper DS (2005): Amiodarone and the thyroid. Am J Med; 118:706-714.

Bellance N, Lestienne P, Rossignol R (2009): Mitochondria: from bioenergetics to the metabolic regulation of carcinogenesis. Front Biosci; 14:4015-4034.

Bellon A, Perez-Garcia G, Coverdale JH, Chacko RC (2009): Seizures associated with levofloxacin: Case presentation and literature review. Eur J Clin Pharmacol; 65 (10):959-962.

Bennett BD, Jetton TL, Ying G, Magnuson MA, Piston DW (1996): Quantitative subcellular imaging of glucose metabolism within intact pancreatic islets. J Biol Chem; 271:3647–3651.

Beste LA, Mersfelder TL (2005): Hyperglycemia and gatifloxacin: a case report and summary of current literature. Am J Geriatr Pharmacother; 3(4):262-265.

Biggs WS (2003): Hypoglycemia and Hyperglycemia Associated with Gatifloxacin Use in Elderly Patients. J Am Board Fam Pract; 16:455-457.

Bischoff U, Schmidt C, Netzer R, Pongs O (2000): Effects of fluoroquinolones on HERG currents. Eur J Pharmacol; 406 (3):341-343.

Blommel AL, Lutes RA (2005): Severe hyperglycemia during renally adjusted gatifloxacin therapy. Ann Pharmacother; 39:1349-1352.

Blondeau JM (1999): A review of the comparative in-vitro activities of 12 antimicrobial agents, with a focus on five new respiratory quinolones. J Antimicrob Chemother; 43 (Suppl B):1-11.

Blum MD, Graham DJ, McCloskey CA (1994): Temafloxacin syndrome: review of 95 cases. Clin Infect Dis; 18:946-950.

Boonen K, Baggerman G, D'Hertog W, Husson SJ, Overbergh L, Mathieu C, Schoofs L (2007): Neuropeptides of the islets of Langerhans: a peptidomics study. Gen Comp Endocrinol; 152(2-3):231-241.

Borg HLA, Andersson A (1981): Long-term effects of glibenclamide on the insulin production, oxidative metabolism and quantitative ultrastructure of mouse pancreatic islets maintained in tissue culture at different glucose concentrations. Acta Diabetol Lat; 18(1):65-83.

Bowie WR, Willetts V, Jewesson PJ (1989): Adverse reactions in a dose-ranging study with a new long-acting fluoroquinolone, Fleroxacin. Antimicrob Agents Chemother; 33 (10):1778-1782.

Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC. (2005): Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. J Histochem Cytochem; 53(9):1087-1097.

Brunicardi FC, Shavelle DM, Andersen DK (1995): Neural regulation of the endocrine pancreas. Int J Pancreatol; 18(3):177-195.

Bryan J, Vila-Carriles WH, Zhao G, Babenko AP, Aguilar-Bryan L (2004): Toward linking structure with function in ATP-sensitive K^+ channels. Diabetes; 53(3):104-112.

Bucher P, Mathe Z, Bosco D, Andres A, Kurfuerst M, Rämsch-Günther N, Buhler L, Morel P, Berney T (2004): Serva collagenase NB1: a new enzyme preparation for human islet isolation. Transplant Proc; 36(4):1143-1144.

Burris RE, Hebrok M (2007): Pancreatic innervation in mouse development and beta-cell regeneration. Neuroscience; 150(3):592-602.

Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A (2006): The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci (USA); 103(7):2334-2339.

Campanella M, Parker N, Tan CH, Hall AM, Duchen MR (2009): IF (1): setting the pace of the F (1) F (o)-ATP synthase. Trends Biochem Sci; 34(7):343-350.

Campi P, Pichler WJ (2003): Quinolone hypersensitivity. Curr Opin Allergy Clin Immunol; 3(4):275-281.

Carlsson PO, Olsson R, Källskog O, Bodin B, Andersson A, Jansson L (2002): Glucose-induced islet blood flow increase in rats: interaction between nervous and metabolic mediators. Am J Physiol- Endocrinol Metab; 283(3):457-464.

Carter JD, Dula SB, Corbin KL, Wu R, Nunemaker CS (2009): A Practical Guide to Rodent Islet Isolation and Assessment. Biol Proced Online; 11(1):3-31.

Casparian JM, Luchi M, Moffat RE, Hinthorn D (2000): Quinolones and tendon ruptures. Southern Medical Journal; 93(5):488-491.

Catero M (2007): Dysglycemia and fluoroquinolones: are you putting patients at risk? J Fam Pract; 56(2):101-107.

Cegrell L (1968): The occurrence of biogenic monoamines in the mammalian endocrine. Acta Physiol Scand Suppl; 314:40-41.

Chalumeau M, Tonnelier S, D'Athis P, Tréluyer JM, Gendrel D, Bréart G, Pons G (2003): Fluoroquinolone safety in pediatric patients: a prospective, multicenter, comparative cohort study in France. Pediatrics; 111(6 Pt 1):714-719.

Chance B, Baltscheffsky H (1958): Respiratory enzymes in oxidative phosphorylation: VII. Binding of intramitochondrial reduced pyridine nucleotide. J Biol Chem; 233(3):736-739.

Chance B, Schoener B, Oshino R, Itshak F, Nakase Y (1979): Oxidation–reduction ratio studies of mitochondria in freeze-trapped samples. NADH and flavoprotein fluorescence signals. J Biol Chem; 254:4764-4771.

Charollais A, Gjinovci A, Huarte J, Bauquis J, Nadal A, Martín F, Andreu E, Sánchez-Andrés JV, Calabrese A, Bosco D, Soria B, Wollheim CB, Herrera PL, Meda P (2000): Junctional communication of pancreatic β cells contributes to the control of insulin secretion and glucose tolerance. J Clin Invest; 106(2):235-243.

Childs SG (2007): Pathogenesis of tendon rupture secondary to fluoroquinolone therapy. Orthopaedic Nursing; 26 (3):175-182.

Civelek VN, Deeney JT, Shalosky NJ, Tornheim K, Hansford RG, Prentki M, Corkey BE (1996): Regulation of pancreatic B-cell mitochondrial metabolism: influence of Ca^{2+}, substrate and ADP. Biochl J; 318:615–621.

Clark DW, Layton D, Wilton LV, Pearce GL, Shakir SA (2001): Profiles of hepatic and dysrhythmic cardiovascular events following use of fluoroquinolone antibacterials: experience from large cohorts from the Drug Safety Research Unit Prescription- Event Monitoring database. Drug Safety; 24:1143-1154.

Coban S, Ceydilek B, Ekiz F, Erden E, Soykan I (2005): Levofloxacin-induced acute fulminant hepatic failure in a patient with chronic hepatitis B infection. Ann Pharmacother; 39(10):1737-1740.

Collombat P, Hecksher-Sørensen J, Serup P, Mansouri A (2006): Specifying pancreatic endocrine cell fates. Mech Dev; 123(7):501-512.

Committee on Infectious Diseases (2006): The Use of Systemic Fluoroquinolone. Pediatrics; 118(3):1287-1292.

Corrao G, Botteri E, Bertù L, Zambon A, Favilli S (2006): Exploring the effect of transient exposure on the risk of acute events by means of time-window designs: an application to fluoroquinolone antibacterials and arrhythmia. Pharmacoepidemiol Drug Safety; 15:31-37

Coupland RE (1958): The innervation of pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. J Anat; 92(1):143-149.

Cui YF, Ma M, Wang ZD, Zhang L, Hu ZL, Han DE (2005): Prevention of central cell damage to isolated islets of Langerhans in hamsters by low temperature preconditioning. Hepatobiliary Pancreat Dis Int; 4(1):139-143.

Darzynkiewicz Z, Staiano-Coico L, Melamed MR (1981): Increased mitochondrial uptake of rhodamine 123 during lymphocyte stimulation. PNAS; 78:2383-2387.

Davis H, McGoodwin E, Reed TG (1989): Anaphylactoid reactions reported after treatment with ciprofloxacin. Ann Intern Med; 111(12):1041-1043.

de Haan BJ, Faas MM, Spijker H, van Willigen JW, de Haan A, de Vos P (2004): Factors influencingi of functional pancreatic rat islets. Panceras; 29(1):15-22.

Detimary P, Van den Berghe G, Henquin JC (1996): Concentration dependence and time course of the effects of glucose on adenine and guanine nucleotides in mouse pancreatic islets. J Biol Chem; 271:20559-20565.

Detimary P, Gilon P, Nenquin M, Henquin JC (1994): Two sites of glucose control of insulin release with distinct dependence on the energy state in pancreatic B-cells. Biochem J; 297 (3):455-461.

Di Virgilio F, Steinburg T, Silverstein S (1990): Inhibition of Fura-2 sequestration and secretion with organic anion transport blockers. Cell Calcium; 11:57-62.

Domagala JM (1994): Structure-activity and structure-side-effect relationships for the quinolone antibacterials. J Antimicrob Chemother; 33(4):685-706.

Donaldson AR, Vandiver JR, Finch CK (2004): Possible gatifloxacin-induced hyperglycemia. Ann Pharmacother; 38(4):602-605.

Douglas WW, Rubin RP (1963): The mechanism of catecholamine release from the adrenal medulla and the role of calcium in stimulus-secretion coupling. J Physiol; 167:288-310.

Doussau de Bazignan A, Thiessard F, Miremont-Salame G, Conri C, Haramburu F (2006): Fluoroquinolone psychiatric adverse effects: review of cases from the french pharmacovigilance database. Rev Med Interne; 27 (6):448-452.

Drossou-Agakidou V, Roilides E, Papakyriakidou-Koliouska P, Agakidis C, Nikolaides N, Sarafidis K, Kremenopoulos G (2004): Use of ciprofloxacin in neonatal sepsis: lack of adverse effects up to one year. Pediatr Infect Dis J; 23(4):346-349.

Dryselius S, Grapengiesser E, Hellman B, Gylfe E (1999): Voltage dependent entry and generation of slow Ca^{2+} oscillations in glucose-stimulated pancreatic beta-cells. Am J Physiol-Endocrinol Metab; 276(3 Pt 1):E512-518.

Duchen MR, Surin A, Jacobson J (2003): Imaging mitochondrial function in intact cells. Methods Enzymol; 361:353-389.

Duchen MR, Smith PA, Ashcroft FM (1993): Substrate-dependent changes in mitochondrial function, intracellular free calcium concentration and membrane channels in pancreatic β-cells. Biochem J; 294 (1):35-42.

Düfer M, Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G (2004): Oscillations of membrane potential and cytosolic Ca^{2+} concentration in SUR1 (-/-) β cells. Diabetologia; 47:488-498.

Duewelhenke N, Krut O, Eysel B (2007): Influence on mitochondria and cytotoxicity of different antibiotics administered in high concentrations on primary human osteoblasts and cell lines. Antimicrob Agents Chemother; 51(1):54-63.

Durey A, Baek YS, Park JS, Lee K, Ryu JS, Lee JS, Cheong MH (2010): Levofloxacin-induced Achilles tendinitis in a young adult in the absence of predisposing conditions. Yonsei Med J; 51(3):454-456.

Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J (2004): Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. Diabetes; 53(7):1714-1720.

Dyachok O, Idevall-Hagren O, Sågetorp J, Tian G, Wuttke A, Arrieumerlou C, Akusjärvi G, Gylfe E, Tengholm A (2008): Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. Cell Metabolism; 8(1):26-37.

Edwin N, Leigh CM (1999): Immunocytochemical identification of islet cells containing calcitonin gene-related peptide-like immunoreactivity in the plains rat pancreas (Pseudomys australis). Singapore Med J; 40:528-530.

Efanova IB, Zaitsev SV, Zhivotovsky B, Kohler M, Efendic S, Orrenius S, Berggren PO (1998): Glucose and tolbutamide induce apoptosis in pancreatic beta-cells: a process dependent on intracellular Ca^{2+} concentration. J Biol Chem; 273:33501-33507.

Elayat AA, el-Naggar MM, Tahir M (1995): An Immunocytochemical and morphometric study of the rat pancreatic islets. J Anat; 186:629-637.

European Medicines Agency (2008): European Medicines Agency recommends restricting the use of oral moxifloxacin containing medicines [press release]. London: European Medicines Agency Press Office, 2008 Jul 24 [online]. Available from URL: http://www.ema.europa.eu/pdfs/human/press/pr/38292708en.pdf
[Accessed 07 June 2010]

Falagas ME, Rafailidis PI, Rosmarakis ES (2007): Arrhythmias associated with fluoroquinolone therapy. Int J Antimicrob Agents; 29 (4): 374-379

Farrington J, Stoudemire A, Tierney J (1995): The role of ciprofloxacin in a patient with delirium due to multiple etiologies. Gen Hosp Psychiatry; 17(1):47-53.

Fish DN, North DS (2001): Gatifloxacin, an advanced 8-methoxy fluoroquinolone. Pharmacother; 21:35-59.

Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB (2005): β-cell secretory products activate α-cell ATP-dependent potassium channels to inhibit glucagon release. Diabetes; 54:1808 –1815.

Fridolf T, Sundler F, Ahrén B (1992): Pituitary adenylate cyclase-activating polypeptide (PACAP): occurrence in rodent pancreas and effects on insulin and glucagon secretion in the mouse. Cell Tissue Res; 269(2):275-279.

Friedrich LV, Dougherty R (2004): Fatal hypoglycemia associated with levofloxacin. Pharmacother; 24:1807-1812.

Frothingham R (2005): Glucose homeostasis abnormalities associated with use of gatifloxacin. CID; 41(9):1269-1276.

Furuhama K (2007): Toxicology assessment of fluoroquinolones: a case study. In: Non clinical drug safety assessment: Practical considerations for successful registration. Sietsema, W. K. and Schwen, R. eds., FDA news, Virginia:571-588.

Gallagher M, Weinberg R, Simpson MV (1986): Effect of the bacterial DNA gyrase inhibitors, novobiocin, nalidixic acid, and oxolinic acid, on oxidative phosphorylation. J Biol Chem; 261(19):8604-8607.

Garber SM, Pound MW, Miller SM (2009): Hypoglycemia associated with the use of levofloxacin. Am J Health Syst Pharm; 66(11):1014-1019.

Gautam D, Jeon J, Li JH, Han SJ, Hamdan FF, Cui Y, Lu H, Deng C, Gavrilova O, Wess J (2008): Metabolic roles of the M3 muscarinic acetylcholine receptor studied with M3 receptor mutant mice: a review. J Recept Signal Transduct Res; 28(1-2):93-108.

Gavin III JR, Kubin R, Choudhri S, Kubitza D, Himmel H, Gross R , Meyer JM (2004): Moxifloxacin and glucose homeostasis: a pooled-analysis of the evidence from clinical and postmarketing studies. Drug Safety; 27:671-686.

Gaynes R, Rimland D, Killum E, Lowery HK, Johnson II TM, Killgore G, Tenover FC (2004): Outbreak of Clostridium difficile infection in a long-term care facility: Association with gatifloxacin use. CID; 38 (5):640-645.

Ge DT, Law PY, Kong SK, Ho YY (2009): Disturbance of cellular glucose transport by two prevalently used fluoroquinolone antibiotics ciprofloxacin and levofloxacin involves glucose transporter type 1. Toxicol Letter; 184(2):81-84.

Ge DT, Law PY, Wong HY, Ho YY (2007): Gatifloxacin affects GLUT1 gene expression and disturbs glucose homeostasis in vitro. Eur J Pharmacol; 573:70-74.

Gembal M, Gilon P, Henquin JC (1992): Evidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. J Clin Invest; 89(4):1288-1295.

Ghaly H, Kriete C, Sahin S, Pfloeger A, Holzgrabe U, Zünkler BJ, Rustenbeck I (2009): The insulinotropic effect of fluoroquinolones. Biochem Pharmacol; 77:1040-1052.

Gilon P, Henquin JC (1992): Influence of membrane potential changes on cytoplasmic Ca^{2+} concentration in an electrically excitable cell, the insulin-secreting pancreatic B-cell. J Biol Chem; 267(29):20713-20720.

Gilon P, Henquin JC (2001): Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. Endocr Rev; 22:565-604.

Gilon P, Jonas JC, Henquin JC (1994): Culture duration and conditions affect the oscillations of cytoplasmic calcium concentration induced by glucose in mouse pancreatic islets. Diabetologia; 37:1007-1014.

Giordano E, Cirulli V, Bosco D, Rouiller D, Halban P, Meda P (1993): B-cell size influences glucose-stimulated insulin secretion. Am J Physiol-Cell Physiol; 265:C358-364.

Gold L, Igra H (2003): Levofloxacin-induced tendon rupture: A case report and review of the literature. J Am Board Fam Pract; 16(5):458-460.

Goligorsky M, Loftus D, Elson E (1986): Alpha l-adrenergic stimulation and cytoplasmic free calcium concentration in cultures renal proximal tubular cells: evidence for compartmentalization of quin-2 and fura-2. J. Cell Physiol; 128:466-474.

Gopel S, Kanno T, Barg S, Galvanovskis J, Rorsman P (1999): Voltage gated and resting membrane currents recorded from B-cells in intact mouse pancreatic islets. J Physiol; 521(3):717-728

Gotoh M, Maki T, Kiyoizumi T, Satomi S, Monaco AP (1985): An improved method for isolation of mouse pancreatic islets. Transplantation; 40:437- 438.

Gottschalk AW, Bachman JW (2009): Death following bilateral complete Achilles tendon rupture in a patient on fluoroquinolone therapy: a case report. J Med Case Reports; 3:1.

Grady RW (2005): Systemic quinolone antibiotics in children: a review of the use and safety. Expert Opin Drug Saf; 4(4):623-630.

Grasela DM (2000): Clinical pharmacology of gatifloxacin, a new fluoroquinolone. CID; 31 (2):S51-58.

Graumlich JF, Habis S, Avelino RR, Salverson SM, Gaddamanugu M, Jamma K, Aldag JC (2005): Hypoglycemia in inpatients after gatifloxacin or levofloxacin therapy: nested case-control study. Pharmacother; 25:1296-1302.

Gribble FM, Tucker SJ, Haug T, Ashcroft FM (1998): MgATP activates the β cell KATP channel by interaction with its SUR1 subunit. Proc Natl Acad Sci (USA); 95 (12):7185-7190.

Grosse-Lackmann T, Zünkler BJ, Rustenbeck I (2003): Specificity of nonadrenergic imidazoline binding sites in insulin-secreting cells and relation to the block of ATP-sensitive K-channels. Ann N Y Acad Sci; 1009:371-377.

Grynkiewicz G, Poenie M, Tsien RY (1985): A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem; 260:3440-3450.

Haerian H, McHugh P, Brown R, Somes G, Solomon SS (2008): Gatifloxacin produces both hypoglycemia and hyperglycemia: a retrospective study. Am J Med Sci; 335:95-98.

Hager JL, Mir MR, Hsu S (2009): Fluoroquinolone-induced generalized fixed drug eruption. Dermatol Online J; 15(12):8.

Hall IH, Schwab UE., Ward ES, Ives TJ (2003): Disposition and intracellular levels of moxifloxacin in human THP-1 monocytes in unstimulated and stimulated conditions. Int J Antimicrob Agents; 22(6):579-587.

Happe MR, Mulhall BP, Maydonovitch CL, Holtzmuller KC (2004): Gatifloxacin-induced hypoglycaemia. Ann Intern Med; 141:968-969.

Hartnell NR, Wilson JP (2004): Replication of the Weber effect using postmarketing adverse event reports voluntarily submitted to the United States Food and Drug Administration. Pharmacotherapy; 24:743-749.

Haruhara N, Niwa H, Kitayama S, Sakai N (2000): Two cases of drug-induced hypoglycemic coma. Journal of Tomakomai City General Hospital; 14:45-48.

Haspel D, Krippeit-Drews P, Aguilar-Bryan L, Bryan J, Drews G, Düfer M (2005): Crosstalk between membrane potential and cytosolic Ca^{2+} concentration in β cells from $Sur1^{-/-}$ mice. Diabetologia; 48(5):913-921.

Haverkamp W, Breithardt G, Camm AJ, Janse MJ, Rosen MR, Antzelevitch C, Escande D, Franz M, Malik M, Moss A, Shah R (2000): The potential for QT prolongation and proarrhythmia by non-antiarrhythmic drugs: clinical and regulatory implications. Eur Heart J; 21:1216–1231.

Health Canada (2010): Updated Labelling for Antibiotic Avelox (Moxifloxacin) Regarding Rare Risk of Severe Liver Injury. 22 March 2010 [online] Available from URL: http://www.hc-sc.gc.ca/ahc-asc/media/advisories-avis/_2010/2010_42-eng.php
[Accessed 01 June 2010].

Henann NE, Zambie MF (2001): Gatifloxacin-associated acute hepatitis. Pharmacotherapy; 21:1579-1582.

Henquin JC (1988): ATP-sensitive K^+ channels may control glucose-induced electrical activity in pancreatic B-cells. Biochem Biophys Res Commun; 156(2):769-775.

Henquin JC (2000): Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes; 49(11):1751-1760.

Henquin JC (2004): Pathways in β-cell stimulus-secretion coupling as targets for therapeutic insulin secretagogues. Diabetes; 53(3):S48-58.

Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y (2010): Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol Rev; 90(1):291-366.

Highsmith S, Bloebaum P, Snodowne KW (1986): Sarcoplasmic reticulum interacts with the Ca (2+) indicator precursor fura-2-am. Bioch Biophys Res Comm; 138:1153-1162.

Hiriart M, Aguilar-Bryan L (2008): Channel regulation of glucose sensing in the pancreatic β-cell, Review. Am J Physiol- Endocrinol Metab; 295:1298-1306.

Ho CC, Chen YC, Hu FC, Yu CJ, Yang PC, Luh KT (2009): Safety of fluoroquinolone use in patients with hepatotoxicity induced by anti-tuberculosis regimens. CID; 48 (11):1526-1533.

Hookman P, Barkin JS (2009): Clostridium difficile associated infection, diarrhea and colitis. World J Gastroenterol; 15(13):1554-1580.

Hori S (2009): Convulsant activity of sitafloxacin and its interactions with anti-inflammatory drugs in mice. J Infect Chemother; 15(4):266-268.

Hunt M (2007): Levofloxacin: dysglycemia and liver disorders. Canadian Adverse Reaction Newsletter; 17(1):1-2.

Husson TR, Mallik AK, Zhang JX, Issa NP (2007): Functional imaging of primary visual cortex using flavoprotein autofluorescence. J Neurosci; 27(32):8665-8675.

Hutton JC (1994): Insulin secretory granule biogenesis and the proinsulin-processing endopeptidases. Diabetologia; 37:S48-56.

Idowu OR, Peggins JO (2004): Simple, rapid determination of enrofloxacin and ciprofloxacin in bovine milk and plasma by high-performance liquid chromatography with fluorescence detection. J Pharm Biomed Anal; 35(1):143-153.

Inagaki N, Gonoi T, Clement JP IV, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S , Bryan J (1995a): Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. Science; 270**:**1166–1170.

Inagaki N, Tsuura Y, Namba N, Masuda K, Gonoi T, Horie M, Seino Y, Mizuta M , Seino S (1995b): Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle and heart. J Biol Chem; 270:5691–5694.

Isenmann R, Friess H, Schlegel P, Fleischer K, Büchler MW (1994): Penetration of ciprofloxacin into the human pancreas. Infection; 22(5):343-346.

Ishihara H, Maechler P, Gjinovci A, Herrera PL, Wollheim CB (2003): Islet β-cell secretion determines glucagon release from neighbouring alpha-cells. Nat Cell Biol; 5(4):330-335.

Ishiwata Y, Itoga Y, Yasuhara M (2006a): Effect of levofloxacin on serum glucose concentration in rats, European. J Pharmacol; 551:168-174.

Ishiwata Y, Sanada, Y, Yasuhara M (2006b): Effects of gatifloxacin on serum glucose concentration in normal and diabetic rats. Biol Pharm Bull; 29:527-531.

Ivarsson R, Quintens R, Dejonghe S, Tsukamoto K, in't Veld P, Renström E, Schuit FC (2005): Redox control of exocytosis: regulatory role of NADPH, thioredoxin, and glutaredoxin. Diabetes; 54(7):2132-2142.

Iwase M, Uchizono Y, Nohara S, Sasaki N, Sonoki K, Iida M (2009): Angiotensin II type 1 receptor antagonists prevent glucose-induced increases in islet blood flow in rats. Scand J Clin Lab Invest; 69(1):145-150.

Jain R, Lammert E (2009): Cell-cell interactions in the endocrine pancreas. Diabetes Obes Metab; 11(4):159-167.

Jain S, Agarwal J, Laskar S, Gupta T, Shrivastava S (2008): Radiation recall dermatitis with gatifloxacin: A review of literature. J Med Imaging Radiat Oncol; 52 (2):191-193.

Jakob I, Hauser IA, Thévenod F, Lindemann B (1998): MDR1 in taste buds of rat vallate papilla: functional, immunohistochemical and biochemical evidence. Am J Physiol- Cell Physiol; 274:182-191.

Jansson L, Bodin B, Källskog O (2008): Glucose-induced time-dependent potentiation of insulin release, but not islet blood perfusion, in anesthetized rats. Ups J Med Sci; 113(1):47-56.

Jansson L, Kullin M, Karlsson FA, Bodin B, Hansen JB, Sandler S (2003): K(ATP) channels and pancreatic islet blood flow in anesthetized rats: increased blood flow induced by potassium channel openers. Diabetes; 52(8):2043-2048.

Järhult J, Holst J (1979): The role of the adrenergic innervation to the pancreatic islets in the control of insulin release during exercise in man. Pflugers Arch; 383(1):41-45.

Jick SS, Jick H, Dean AD (1993): A follow-up safety study of ciprofloxacin users. Pharmacotherapy; 13:461-464.

Jongen-Lavrencic M, Schneeberger PM, van der Hoeven JG (2003): Ciprofloxacin-induced toxic epidermal necrolysis in a patient with systemic lupus erythematosus. Infection; 1(6):428-429.

Jörns A, Munday R, Tiedge M, Lenzen S (1997): Ultrastructural changes in isolated incubated ob/ob mouse pancreatic islets induced by alloxan, various alloxan derivatives and ninhydrin. J Endocrin; 155:283-293.

Jose J, Jimmy B, Saravu K (2007): Dysglycemia associated with the use of fluoroquinolones-focus on gatifloxacin. Journal of Clinical and Diagnostic Research; 3:185-187.

Kamisawa T (2004): Clinical significance of the minor duodenal papilla and accessory pancreatic duct. J Gastroenterol; 39(7):605-615.

Kang J, Wang L, Chen XL, Triggle DJ, Rampe D (2001): Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K^+ channel HERG. Mole Pharmacol; 59 (1):122-126.

Kangas L, Grönroos M, Nieminen AL (1984): Bioluminescence of cellular ATP: A new method for evaluating cytotoxic agents in vitro. Med. Biol; 62:338-343.

Kashida Y, Kato M (1997): Characterization of fluoroquinolone-induced Achilles tendon toxicity in rats: comparison of toxicities of 10 fluoroquinolones and effects of anti-inflammatory compounds. Antimicrob Agents Chemother; 41:2389–2393.

Kato M (2008): Chondrotoxicity of quinolone antimicrobial agents. Journal of Toxicologic Pathology; 21 (3):123-131.

Kaufman MB, Stoukides CA, Campbell NA (1994): Physicians' liability for adverse drug reactions. South Med J; 87(8):780-784.

Kelesidis T, Canseco E (2010): Quinolone-induced hypoglycemia: a life-threatening but potentially reversible side effect. Am J Med; 123(2): 5-6.

Kennedy ED, Rizzuto R, Theler JM, Pralong WF, Bastianutto C, Pozzan T, Wollheim CB (1996): Glucose-stimulated insulin secretion correlates with changes in mitochondrial and cytosolic Ca^{2+} in aequorin-expressing INS-1 cells. J Clin Invest; 98(11):2524-2538.

Kernt M, Neubauer AS, Liegl RG, Lackerbauer CA, Eibl KH, Alge CS, Ulbig MW, Kampik AA (2009): Intracameral moxifloxacin: In vitro safety on human ocular cells Cornea; 28 (5):553-561.

Khaira A, Gupta A, Tandon N, Agarwal SK (2009): Gatifloxacin-induced severe hyperglycaemia and ketoacidosis in a non-diabetic renal transplant recipient. Clin Exp Nephrol.; 13(1):89-91.

Khaliq Y, Zhanel GG (2005): Musculoskeletal injury associated with fluoroquinolone antibiotics. Clin Plast Surg; 32 (4):495-502

Khovidhunkit W, Sunthornyothin S (2004): Hypoglycemia, hyperglycemia, and gatifloxacin. Ann Intern Med; 141(12):969.

Kim A, Miller K, Jo J, Kilimnik G, Wojcik P, Hara M (2009): Islet architecture: a comparative study. Islets; 1:129-136.

Kinard TA, Satin LS (1996): Temperature modulates the Ca^{2+} current of HIT-T15 and mouse pancreatic β-cells. Cell Calcium; 20 (6):475-482.

Kindmark H, Kohler M, Brown G, Branstrom R, Larsson O, Berggren PO (2001): Glucose-induced oscillations in cytoplasmic free Ca^{2+} concentration precede oscillations in mitochondrial membrane potential in the pancreatic β-cell. J Biol Chem; 276:34530–34536.

Klecak G, Urbach F, Urwyler H (1997): Fluoroquinolone antibacterials enhance UVA-induced skin tumours. J Photochem Photobio; 37(3):174-181.

Kobayashi N, Tsuhako Y, Matsuyama R, Fujinaga M, Nakamoto M (1991): Hypoglycemic attacks in 3 patients with non-diabetic chronic renal failure on hemodialysis, with discussion of a patient who showed enoxacin-induced hypoglycemia. J Jpn Soc Dial Ther; 24:951-956.

Kowatari K, Nakashima K, Ono A, Yoshihara M, Amano M, Toh S (2004): Levofloxacin-induced bilateral Achilles tendon rupture: a case report and review of the literature. J Orthop Sci; 9:186-190.

Krippeit-Drews P, Düfer M, Drews G (2000): Parallel oscillations of intracellular calcium activity and mitochondrial membrane potential in mouse pancreatic B-cells. Biochem Biophys Res Commun; 267(1):179-183.

Kushner JM, Peckman HJ, Snyder CR (2001): Seizures associated with fluoroquinolones. Ann Pharmacother; 35(10):1194-1198.

Lacy PE, Kostianovsky M (1967): Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes; 16:35-39.

LaSalvia EA, Domek GJ, Gitlin DF (2010): Fluoroquinolone-induced suicidal ideation. Gen Hosp Psychiatry; 32(1):108-110.

Lawrence KR, Adra M, Keir C (2006): Hypoglycemia-induced anoxic brain injury possibly associated with levofloxacin. J Infect; 52 (6):177-180.

LeBlanc M, Belanger C, Cossette P (2004): Severe and resistant hypoglycemia associated with concomitant gatifloxacin and glyburide therapy. Pharmacotherapy; 24:926-931.

Lehmann R, Zuellig RA, Kugelmeier P, Baenninger PB, Moritz W, Perren A, Clavien PA, Weber M, Spinas GA (2007): Superiority of small islets in human islet transplantation. Diabetes; 56(3):594-603.

Leibovitz E (2006): The use of fluoroquinolones in children. Curr Opin Pediatr; 18:64-70.

Lenzen S, Lerch M, Peckmann T, Tiedge M (2000): Differential regulation of $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets by glucose, alpha-ketoisocaproic acid, glyceraldehyde and glycolytic intermediates. Biochim Biophys Acta; 1523:65-72.

Lenzen S, Peckmann T (2001): Effects of tolbutamide and N-benzoyl-D-phenylalanine (NBDP) on the regulation of $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets. Biochem Pharmacol; 62:923-928.

Leone R, Venegoni M, Motola D, Moretti U, Piazzetta V, Cocci A, Resi D, Mozzo F, Velo G, Burzilleri L, Montanaro N, Conforti A (2003): Adverse drug reactions related to the use of fluoroquinolone antimicrobials: an analysis of spontaneous reports and fluoroquinolone consumption data from 3 Italian regions. Drug Saf; 26(2):109-120.

Lernmark A (1974): The preparation of, and studies on, free cell suspension from mouse pancreatic islets. Diabetologia; 10:431-438.

Lewis-Hall F (2006): Tequin (gatifloxacin): full prescribing and important safety information. Princeton, N.J.: Bristol-Myers Squibb, February 15, 2006. Avaible from URL: http://www.rxlist.com/tequin-drug.htm [Accessed 30.04.2010].

Li Y, Liu L, Li J, Xie L, Wang GJ, Liu XD (2009): Transport of gatifloxacin involves Na^{+}/Ca^{2+} exchange and excludes P-glycoprotein and multidrug resistance associated-proteins in primary cultured rat brain endothelial cells. Eur J Pharmacol; 616(1-3):68-72.

Lin G, Hays DP, Spillane L (2004): Refractory hypoglycemia from ciprofloxacin and glyburide interaction. J Toxicol Clin Toxicol; 42(3):295-297.

Ling Z, Pipeleers DG (1994): Preservation of glucose-responsive islet β-cells during serum-free culture. Endocrinol; 134(6):2614-2621.

Lipsky BA, Baker CA (1999): Fluoroquinolone toxicity profiles: A review focusing on newer agents.CID; 28 (2):352-364.

Lipsky BA, Giordano P, Choudhri S, Song J (2007): Treating diabetic foot infections with sequential intravenous to oral moxifloxacin compared with piperacillin-tazobactam/amoxicillin-clavulanate. J Antimicrob Chemother; 60:370-376.

Lodise T, Graves J, Miller C, Mohr JF, Lomaestro B, Smith RP (2007): Effects of gatifloxacin and levofloxacin on rates of hypoglycemia and hyperglycemia among elderly hospitalized patients. Pharmacother; 27(11):1498-1505.

Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, Bourgault AM, Nguyen T, Frenette C, Kelly M, Vibien A, Brassard P, Fenn S, Dewar K, Hudson TJ, Horn R, René P, Monczak Y, Dascal A (2005): A predominantly clonal multi-institutional outbreak of Clostridium difficile–associated diarrhea with high morbidity and mortality. N Engl J Med; 353:2442-2449.

Loo KC, Cario AC, Zhang F, Walters JD (1997): Regulation of ciprofloxacin uptake in human promyelocytic leukemia cells and polymorphonuclear leukocytes. J Leukoc Biol; 61:619-623.

Lowes DA, Wallace C, Murphy MP, Webster NR, Galley HF (2009): The mitochondria targeted antioxidant MitoQ protects against fluoroquinolone-induced oxidative stress and mitochondrial membrane damage in human Achilles tendon cells. Free Radic Res; 43(4):323-328.

MacDonald MJ, Fahien LA, Brown LJ, Hasan NM, Buss JD, Kendrick MA (2005): Perspective: emerging evidence for signaling roles of mitochondrial anaplerotic products in insulin secretion. Am J Physiol- Endocrinol Metabol; 288:E1-15.

MacDonald PE, Ha XF, Wang J, Smukler SR, Sun AM, Gaisano HY, Salapatek AM, Backx PH, Wheeler MB (2001): Members of the Kv1 and Kv2 voltage-dependent K ($^{+}$) channel families regulate insulin secretion. Mol Endocrinol; 15:1423-1435.

Maechler P (2002): Mitochondria as the conductor of metabolic signals for insulin exocytosis in pancreatic β cells. Cell Mol Life Sci; 59(11):1803-1818.

Maeda N, Tamgawa T, Niki I, Miura H, Ozawa K, Watanabe G (1996): Increase in insulin release from rat pancreatic islets by quinolone antibiotics. Br J Pharmacol; 117:372-376.

Malik M, Hnatkova K, Schmidt A, Smetana P (2009): Electrocardiographic QTc changes due to moxifloxacin infusion. J Clin Pharmacol; 49:674-683.

Mammano F, Bortolozzi M (2010): Ca^{2+} imaging: Principles of analysis and enhancement. Neuromethods; 43:57-80

Manzini I, Schild D (2003): Multidrug resistance transporters in the olfactory receptor neurons of Xenopus laevis tadpoles. J Physiol; 546 (2):375-385.

Marhfour I, Moulin P, Marchandise J, Rahier J, Sempoux C, Guiot Y (2009): Impact of sur1 gene inactivation on the morphology of mouse pancreatic endocrine tissue. Cell and tissue research; 335 (3):505-515

Marutani K, Matsumoto M, Otabe Y, Nagamuta M, Tanaka K, Miyoshi A, Hasegawa T, Nagano H, Matsubara S, Kamide R, Yokota T, Matsumoto F, Ueda Y (1993): Reduced phototoxicity of a fluoroquinolone antibacterial agent with a methoxy group at the 8 position in mice irradiated with long-wavelength UV light. Antimicrob Agents Chemother; 37(10):2217-2223.

Maunz G, Conzett T, Zimmerli W (2009): Cutaneous vasculitis associated with fluoroquinolones. Infection; 37(5):466-468.

McGarvey WC, Singh D, Trevino SG (1996): Partial Achilles tendon ruptures associated with fluoroquinolone antibiotics: a case report and literature review. Foot Ankle Int; 17(8):496-498.

Meda P (1996): The role of gap junction membrane channels in secretion and hormonal action. J Bioenerg Biomembr; 28(4):369-377.

Menzies DJ, Dorsainvil PA, Cunha BA und Johnson DH (2002): Severe and persistent hypoglycaemia due to gatifloxacin interaction with oral hypoglycaemic agents. Am J Med; 113: 332-334.

Milner EP, Zheng Q, Kermode JC (1998): Ristocetin-mediated interaction of human von Willebrand factor with platelet glycoprotein Ib evokes a transient calcium signal: Observations with Fura-PE3. J Lab Clin Med; 131(1):49-62.

Mitchell P, Moyle J (1967): Respiration-driven proton translocation in rat liver mitochondria. Biochem J; 105(3):1147-1162.

Mitscher LA (2005): Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents. Chem Rev; 105:559–592.

Mohr III JF, Peymann PJ, Troxell E, Lodise TP, Ostrosky-Zeichner L (**2008):** Risk factors for hyperglycemia in hospitalized adults receiving gatifloxacin: A retrospective, nested case-controlled analysis. Clin Ther; 30(1):152-157.

Mohr III JF, McKinnon PS, Peymann PJ, Kenton I, Septimus E, Okhuysen PC (2005): A retrospective, comparative evaluation of dysglycemias in hospitalized patients receiving gatifloxacin, levofloxacin, ciprofloxacin, or ceftriaxone. Pharmacotherapy; 25:1303-1309.

Mojet HM, Jacobson DJ, Keelan J, Vergun O, Duchen MR (2001): Monitoring mitochondrial functions in single cells in: Calcium signalling, a practical approach. Tepikin A; Oxford University Press:99-100.

Moore B, Safani M, Keesey J (1988): Possible exacerbation of myasthenia gravis by ciprofloxacin. Lancet; 1(8590):882.

Moreton RB (1994): Optical methods for imaging ionic activities. Scanning Microsc Suppl; 8:371-390.

Moskalewski S (1965): Isolation and culture of the islets of Langerhans of the guinea pig. Gen comp Endocr; 5:342-353.

Mulhall JP, Bergmann LS (1995): Ciprofloxacin-induced acute psychosis. Urology; 46:1102-1103.

Mumford CJ, Ginsberg L (1990): Ciprofloxacin and myasthenia gravis. BMJ; 301(6755):818.

Munro AW, Noble MA (1999): Fluorescence Analysis of Flavoproteins. Methods in Molecular Biology; 131:25-48.

Murray TS, Baltimore RS (2007): Pediatric uses of fluoroquinolone antibiotics. Pediatr Ann; 36:336-342.

Muto CA, Pokrywka M, Shutt K, et al (2005): A large outbreak of Clostridium difficile–associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol; 26:273-280.

Naber KG, Adam D (1998): Classification of fluoroquinolones. Int J Antimicrob Agents; 10: 255-257.

Nadal A, Quesada I, Soria B (1999): Homologous and heterologous asynchronicity between identified α-, β- and γ-cells within intact islets of Langerhans in the mouse. J Physiol; 517:85-93.

Nadal A, Valdeolmillos M, Soria B (1994): $[Ca^{2+}]_i$ changes induced by tolbutamide in single pancreatic islets: In Frontiers of insulin secretion and pancreatic β-cell research. Flatt P, Lenzen S. Eds. London and Nigati-shi, Japan, Smith- Gordon/Nishimura:237-241.

Nagai M, Nagata S, Yamagishi N, Satoh H, Furuhama K (2010): Clinicopathological aspect of dysglycemia in naive and diabetic rats Induced by the fluoroquinolone antibacterial gatifloxacin. J Vet Med Sci; 72(5):567-573.

Namkung Y, Skrypnyk N, Jeong M-J, Lee T, Lee MS, Kim HL, Chin H, Suh PG, Kim SS, Shin HS (2001): Requirement for the L-type Ca^{2+} channel alpha1D subunit in postnatal pancreatic beta cell generation. J Clin Invest; 108:1015-1022.

Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domecq C, Greenblatt DJ (1981): A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther; 30:239-245.

Navarro-Tableros V, Fiordelisio T, Hernández-Cruz A, Hiriart M (2007): Physiological development of insulin secretion, calcium channels, and GLUT2 expression of pancreatic rat β-cells. Am J Physiol-Endocrinol Metab; 292: E1018-1029.

Nenquin M, Szollosi A, Aguilar-Bryan L, Bryan J, Henquin JC (2004): Both triggering and amplifying pathways contribute to fuel-induced insulin secretion in the absence of sulfonylurea receptor-1 in pancreatic β-cells. J. Biol Chem; 279(31):32316-32324.

Niwa K, Shibuya I, Kanno T (1996): Temperature dependence of processes proximal and distal to the glucose-induced $[Ca^{2+}]_i$ rise in stimulation-secretion coupling in rat pancreatic islets. Biological Signals; 5:30-43.

Novell MJ, Morreale CA (2010): The relationship between inpatient fluoroquinolone use and Clostridium difficile-associated diarrhea. Ann Pharmacother; 44(5):826-831.

Nunemaker CS, Satin LS (2004): Comparison of metabolic oscillations from mouse pancreatic β cells and islets. Endocrine; 25(1):61-67.

Nunemaker CS, Zhang M, Satin LS (2004): Insulin feedback alters mitochondrial activity through an ATP-sensitive K^+ channel-dependent pathway in mouse islets and β-cells. Diabetes; 53(7):1765-1772.

Nunemaker CS, Zhang M, Wasserman DH, McGuinness OP, Powers AC, Bertram R, Sherman A, Satin LS (2005): Individual mice can be distinguished by the period of their islet calcium oscillations: is there an intrinsic islet period that is imprinted in vivo? Diabetes 54: 3517-3522.

Nyman LR, Ford E, Powers AC, Piston DW (2010): Glucose-dependent blood flow dynamics in murine pancreatic islets in vivo. Am J Physiol- Endocrinol Metab; 298(4):E807-814.

Oliphant CM, Green GM (2002): Quinolones: A comprehensive review. Am Fam Physician; 65:455-464.

Onyenwenyi AJ, Winterstein AG, Hatton RC (2008): An evaluation of the effects of gatifloxacin on glucose homeostasis. Pharm World Sci; 30(5):544-549.

Orci L, Ravazzola M, Amherdt M, Madsen O, Perrelet A, Vassalli J-D, Anderson GW (1986): Conversion of proinsulin to insulin occurs coordinately with acidification of maturing secretory vesicles. J Cell Biol; 103:2273-2281.

Orlander JD, Serrao R (2004): Gatifloxacin-induced hyperglycemia. Infectious Diseases in Clinical Practice; 12(4):230-232.

Ouédraogo G, Morlière P, Santus R, Miranda MA, Castell JV (2000): Damage to mitochondria of cultured human skin fibroblasts photosensitized by fluoroquinolones. J Photochem Photobiol B; 58(1):20-25.

Ovartlarnporn M, Jongjaroenprasert W (2007): Advancing age and renal impairment as important predisposing factors of gatifloxacin-induced hyperglycemia in non-diabetes patients. J Med Assoc Thai; 90(3):569-573.

Owens Jr RC (2005): Fluoroquinolone-associated dysglycemias: a tale of two toxicities. Pharmacotherapy; 25(10):1291-1295.

Owens Jr RC, Ambrose PG (2000): Clinical use of the fluoroquinolones. Med Clin North Am; 84:1447-1469.

Owens Jr RC, Ambrose PG (2005): Antimicrobial Safety: Focus on Fluoroquinolones. Clin Infect Dis; 41 (2):144-157.

Ozyuncu O, Nemutlu E, Katlan D, Kir S, Beksac MS (2010): Maternal and fetal blood levels of moxifloxacin, levofloxacin, cefepime and cefoperazone. Int J Antimicrob Agents; 36(2):175-178.

Panten U, Schwanstecher M, Schwanstecher C (1992): Pancreatic and extrapancreatic sulfonylurea receptors. Horm Metab Res; 24(12):549-554.

Panten U, Schwanstecher M, Wallasch A, Lenzen S (1988): Glucose both inhibits and stimulates insulin secretion from isolated pancreatic islets exposed to maximally effective concentrations of sulfonylureas. Naunyn Schmiedebergs Arch Pharmacol; 338(4):459-462.

Panten U, Ishida H, Schauder P, Frerichs H, Hasselblatt A (1977): A versatile microperifusion system. Analytical Biochemistry; 82:317-326.

Panten U, Ishida H (1975): Fluorescence of oxidized flavoproteins from perfused isolated pancreatic islets. Diabetologia; 11:569-573.

Park-Wyllie LY, Juurlink DN, Kopp A, Shah BR, Stukel TA, Stumpo C, Dresser L, Low DE, Mamdani MM (2006): Outpatient gatifloxacin therapy and dysglycemia in older adults. N Engl J Med; 354(13):1352-1361.

Pascual A, Garcia I, Ballestra S, Perea E (1999): Uptake and intracellular activity of moxifloxacin in human neutrophils an tissue cultured epithelium cells. Antimicrob Agents Chemother; 43:12-15.

Patterson GH, Knobel SM, Arkhammar P, Thastrup O, Piston DW (2000): Separation of the glucose-stimulated cytoplasmic and mitochondrial NAD(P)H responses in pancreatic islet β cells. Proc Natl Acad Sci (USA); 97(10):5203-5207.

Patton GS, Ipp E, Dobbs RE, Orci L, Vale W, Unger RH (1977): Pancreatic immunoreactive somatostatin release. Proc Natl Acad Sci (USA); 74:2140-2143.

Perry CM, Barman Balfour JA, Lamb HM (1999): Gatifloxacin. Drugs; 58(4):683-696.

Peters B, Pinching AJ (1989): Fatal anaphylaxis associated with ciprofloxacin in a patient with AIDS related complex. BMJ; 298 (6673): 605.

Pipeleers D, in't Veld P.I, Maes E, Van De Winkel M (1982): Glucose-induced insulin release depends on functional cooperation between islet cells. Proc Natl Acad Sci (USA); 79:7322-7325.

Proks P, Ashcroft F (1997): Phentolamine block of KATP channels is mediated by Kir6.2. Proc Natl Acad Sci (USA); 94: 11716-11720.

Quesada I, Todorova MG, Soria B (2006): Different metabolic responses in alpha-, beta-, and delta-cells of the islet of Langerhans monitored by redox confocal microscopy. Biophys J; 90(7):2641-2650.

Quigley CA, Lederman JR (2004): Possible gatifloxacin-induced seizure. Ann Pharmacother; 38(2):235-237.

Rahier J, Wallon J, Henquin JC (1981): Cell populations in the endocrine pancreas of human neonates and infants. Diabetologia; 20(5):540-546.

Raschi E, Poluzzi E, Zuliani C, Muller A, Goossens H, De Ponti F (2009): Exposure to antibacterial agents with QT liability in 14 European countries: trends over an 8-year period. Br J Clin Pharmacol; 67(1):88-98.

Rauser EH, Ariano RE, Anderson BA (1990): Exacerbation of myasthenia gravis by norfloxacin. DICP; 24 (2):207-208.

Ravier MA, Rutter GA (2010): Isolation and culture of mouse pancreatic islets for ex vivo imaging studies with trappable or recombinant fluorescent probes. Methods Mol Biol; 633:171-184.

Ravier MA, Nenquin M, Miki T, Seino S, Henquin JC (2009): Glucose controls cytosolic Ca^{2+} and insulin secretion in mouse islets lacking adenosine triphosphate-sensitive K^+ channels owing to a knockout of the pore-forming subunit Kir6.2. Endocrinology; 150 (1):33-45.

Ravier MA, Rutter GA (2005): Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic α-cells. Diabetes; 54:1789-1797.

Ravier MA, Sehlin J, Henquin JC (2002): Disorganization of cytoplasmic Ca^{2+} oscillations and pulsatile insulin secretion in islets from ob/ob mice. Diabetologia; 45:1154-1163.

Reeves RR (2007): Exacerbation of psychotic symptoms associated with gatifloxacin. Psychosomatics; 48(1):87.

Reinbothe TM, Ivarsson R, Li DQ, Niazi O, Jing X, Zhang E, Stenson L, Bryborn U, Renström E (2009): Glutaredoxin-1 mediates NADPH-dependent stimulation of calcium-dependent insulin secretion. Mol Endocrinol; 23(6):893-900.

Renström E, Eliasson L, Bokvist K, Rorsman P (1996): Cooling inhibits exocytosis in single mouse pancreatic B-cells by suppression of granule mobilization. J Physiol; 494:41-52.

Roberge RJ, Kaplan R, Frank R, Fore C (2000): Glyburide–ciprofloxacin interaction with resistant hypoglycemia. Ann Emerg Med; 36 (2):160-163.

Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H (2003): Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes; 52(3):581-587.

Roe MW, Lemasters JJ, Herman B (1990): Assessment of Fura-2 for measurements of cytosolic free calcium. Cell Calcium; 11:63-73.

Rollof J, Vinge E (1993): Neurologic adverse effects during concomitant treatment with ciprofloxacin, NSAIDs, and chloroquine: possible drug interaction. Ann Pharmacother; 27(9): 1058-1059.

Roquer J, Cano A, Seoane JL, Pou Serradell A (1996): Myasthenia gravis and ciprofloxacin. Acta Neurol Scand; 94:419-420.

Rorsman P, Berggren PO, Bokvist K, Ericson H, Möhler H, Ostenson CG, Smith PA (1989): Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. Nature; 341(6239):233-236.

Rosignoli MT, Di Loreto G, Dionisio P (2010): Effects of prulifloxacin on cardiac repolarization in Healthy subjects: a randomized, crossover, double-blind versus placebo, moxifloxacin-controlled study. Clin Drug Investig; 30(1):5-14.

Rossi M, Lusini G, Biasella A, Mazzocchio R (2009): Prulifloxacin as a trigger of myasthenia gravis. J Neurol Sci; 280 (1-2):109-110.

Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T (2003): Mitochondrial threshold effects. Biochem J; 370(Pt 3):751-762.

Rouiller D, Schusdziarra V, Unger RH (1981): Insulin inhibits somatostatin-like immunoreactivity release stimulated by intragastric HCl. Diabetes; 30(9):735-738.

Rubinstein E (2001): History of quinolones and their side effects. Chemotherapy; 47 (3):3-8.

Rustenbeck I, Krautheim A, Jörns A, Steinfelder HJ (2004): Beta-cell toxicity of ATP-sensitive K+ channel-blocking insulin secretagogues. Biochem Pharmacol; 67(9):1733-1741.

Rustenbeck I (1999): Stimulus-secretion coupling in pancreatic B-cells: From physiology to pharmacology. Diabetes and Metabolism; 8 (5):217-222.

Rustenbeck I, Herrmann C, Grimmsmann T (1997a): Energetic requirement of insulin secretion distal to calcium influx. Diabetes; 46(8):1305-1311.

Rustenbeck I, Herrmann C, Ratzka P, Hasselblatt A (1997b): Imidazoline/ guanidinium binding sites, and their relation to inhibition of K (ATP) channels in pancreatic B-cells. Naunyn-Schmiedebergs Arch Pharmacol; 356:410-417.

Rutter GA, Leclerc I (2009): The AMP-regulated kinase family: Enigmatic targets for diabetes therapy. Mol Cell Endocrinol; 297(1-2):41-49.

Sabharwal V, Marchant CD (2006): Fluoroquinolone use in children. Pediatr Infect Dis J; 25:257-258.

Salehi A, Qader S.S, Grapengiesser E, Hellman B (2005): Inhibition of purinoceptors amplifies glucose-stimulated insulin release with removal of its pulsatility. Diabetes; 54:2126-2131.

Salvi AE, Metelli GP, Bosco A, Berizzi A, Hacking SA, Cantalamessa A (2007): Spontaneous bilateral Achilles tendon rupture in a patient treated with oral levofloxacin. J Orthop Traumatol; 8 (2):86-90.

Samols E, Marks V, Marri G (1965): Promotion of insulin secretion by glucagon. Lancet; 2:415-416.

Saraya A, Yokokura M, Gonoi T, Seino S (2004): Effects of fluoroquinolones on insulin secretion and β-cell ATP-sensitive K^+ channels. Eur J Pharmacol; 497(1):111-117.

Satin LS (2000): Localized calcium influx in pancreatic β-cells: its significance for Ca^{2+}-dependent insulin secretion from the islets of Langerhans. Endocrine; 13(3):251-262.

Sato Y, Aizawa T, Komatsu M, Okada N, Yamada T (1992): Dual functional role of membrane depolarization/Ca^{2+} influx in rat pancreatic B-cell. Diabetes; 41:438-443.

Savontaus E, Fagerholm V, Rahkonen O, Scheinin M (2008): Reduced blood glucose levels, increased insulin levels and improved glucose tolerance in alpha2A-adrenoceptor knockout mice. Eur J Pharmacol; 578(2-3):359-364.

Scanlon M, Williams DA, Fay FS (1987): A Ca^{2+} -insensitive form of fura-2 associated with polymorphonuclear leukocytes. Assessment and accurate Ca^{2+} measurement. J Biol Chem; 262: 6308-6312.

Scheepers A, Joost HG, Schurmann A (2004): The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. J Parenter Enteral Nutr; 28(5):364-371.

Scherer K, Bircher AJ (2005): Hypersensitivity reactions to fluoroquinolones. Curr Allergy Asthma Rep; 5(1):15-21.

Schmuck G, Schürmann A, Schlüter G (1998): Determination of the excitatory potencies of fluoroquinolones in the central nervous system by an in vitro model. Antimicrob Agents Chemother; 42:1831-1836.

Scholz R, Thurman RG, Williamson JR, Chance B, Bucher T (1969): Flavin and pyridine nucleotide oxidation-reduction changes in perfused rat liver. J Biol Chem; 244: 2317-2324.

Schuchmann S, Kovacs R, Kann O, Heinemann U, Buchheim K (2001): Monitoring NAD(P)H autofluorescence to assess mitochondrial metabolic functions in rat hippocampal-entorhinal cortex slices. Brain Res Protoc; 7: 267-276

Seghers V, Nakazaki M, DeMayo F, Aguillar-Bryan L, Bryan J (2000): Sur1 knockout mice: a model for KATP channel-independent regulation of insulin secretion. J Biol Chem; 275:9270-9277.

Seliger HH, McElroy WD (1960): Spectral emission and quantum yield of firefly bioluminescence. Arch Biochem Biophys; 88:136-141.

Sendzik J, Lode H, Stahlmann R (2009): Quinolone-induced arthropathy: an update focusing on new mechanistic and clinical data. Int J Antimicrob Agents; 33 (3):194-200.

Senthilkumar P, Dinakaran M, Yogeeswari P, China A, Nagaraja V, Sriram D (2009): Antimycobacterial activities of novel fluoroquinolones. Biomed Pharmacother; 63(1):27-35.

Shehab N, Patel PR, Srinivasan A, Budnitz DS (2008): Emergency department visits for antibiotic-associated adverse events. Clin Infect Dis; 47:735-743.

Sherazi S, DiSalle M, Daubert JP, Shah AH (2008): Moxifloxacin-induced torsades de pointes. Cardiol J; 15(1):71-73.

Shuttleworth CW (2010): Use of NAD(P)H and flavoprotein autofluorescence transients to probe neuron and astrocyte responses to synaptic activation. Neurochem Int; 56(3):379-386.

Singh M, Jacob JJ, Kapoor R, Abraham J (2008): Fatal hypoglycemia with levofloxacin use in an elderly patient in the post-operative period. Langenbecks Arch Surg; 393(2):235-238.

Skoglund G, Lundquist I, Ahren B (1986): Effects of alpha 1- and alpha 2-adrenoceptor stimulation and blockade on plasma insulin levels in the mouse. Pancreas; 1:415-420.

Slack JM (1995): Developmental biology of the pancreas. Development; 121 (6):1569-1580.

Smelt MJ, Faas MM, de Haan BJ, de Vos P (2008): Pancreatic beta-cell purification by altering FAD and NAD(P)H metabolism. Exp Diabetes Res; 2008:1-10.

Smith KM, Lomaestro BM (2003): What role do fluoroquinolone antimicrobial agents play in cardiac dysfunction and altered glycemic control? J Pharmacy Practice; 16:349-360.

Spahr L, Rubbia-Brandt L, Marinescu O, Armenian B, Hadengue A (2001): Acute fatal hepatitis related to levofloxacin. J Hepatol; 35(2):308-309.

Spooner BS, Walther BT, Rutter WJ (1970): The development of the dorsal and ventral mammalian pancreas in vivo and in vitro. J Cell Biol; 47 (1):235-246.

Stagner JI, Samols E (1985): Role of intrapancreatic ganglia in regulation of periodic insular secretions. Am J Physiol- Endocrinol Metab; 248(5 Pt 1):522-530.

Stahlmann R (2002): Clinical toxicological aspects of fluoroquinolones. Toxicology Letters; 127:269–277.

Stahlmann R, Kühner S, Shakibaei M, Schwabe R, Flores J, Evander SA, van Sickle DC (2000): Chondrotoxicity of ciprofloxacin in immature beagle dogs: immunohistochemistry, electron microscopy and drug plasma concentrations. Arch Toxicol; 73(10-11):564-572.

Stahlmann R, Schwabe R (1997): Safety profile of grepafloxacin compared with other fluoroquinolones. J Antimicrob Chemother; 40(A):83–92.

Steinberg SF, Bilezikian JP, Al-Awqati Q (1987): Fura-fluorescence is localized to mitochondria in endothelial cells. Am J Physiol- Cell Physiol; 253(5 Pt 1):744-747.

Stevens SX, Fouraker BD, Jensen HG (1991): Intraocular safety of ciprofloxacin. Arch Ophthalmol; 109:1737-1743.

Strevel EL, Kuper A, Gold WL (2006): Severe and protracted hypoglycaemia associated with co-trimoxazole use. Lancet Infect Dis; 6(3):178-182.

Strowski MZ, Kohler M, Chen HY, Trumbauer ME, Li Z, Szalkowski D, Gopal-Truter S, Fisher JK, Schaeffer JM, Blake AD, Zhang BB, Wilkinson HA (2003): Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. Mol Endocrinol; 17(1):93-106.

Suda M, Minami T, Aono H, Koh T, Hamada Y, Yashiro M (2000): Repeated hypoglycemia induced by lomefloxacin in chronic renal failure on hemodialysis. J Kyoto City Hosp; 20: 41-46.

Szollosi A, Nenquin M, Henquin JC (2007): Overnight culture unmasks glucose-induced insulin secretion in mouse islets lacking ATP-sensitive K^+ channels by improving the triggering Ca^{2+} signal. J Biol Chem; 282: 14768-14776.

Thomas PM, Cote GJ, Hallman DM, Mathew PM (1995): Homozygosity mapping to chromosome 11p of the gene for familial persistent hyperinsulinemic hypoglycaemia of infancy. Am J Hum Genet; 56:416-421.

Thomas RJ (1994): Neurotoxicity of antibacterial therapy. South Med J; 87 (9):869-874.

Thompson AM (2007): Ocular toxicity of fluoroquinolones. Clin Experiment Ophthalmol; 35 (6):566-577.

Thorens B, Mueckler M (2010): Glucose transporters in the 21st Century. American J Physiol - Endocrinology and Metabolism; 298 (2): E141-145.

Tokura Y (1998): Quinolone photoallergy: photosensitivity dermatitis induced by systemic administration of photohaptenic drugs. J Dermatol Sci; 18(1):1-10.

Tomita T, Onishi M, Sato E, Kimura Y, Kihira K (2007): Gatifloxacin induces augmented insulin release and intracellular insulin depletion of pancreatic islet cells. Biol Pharm Bull; 30(4):644-647.

Traa BS, Walker CL, Munos M, Black RE (2010): Antibiotics for the treatment of dysentery in children. Int J Epidemiol; 39 (1):70-74.

Tripathi A, Chen SI, O'Sullivan S (2002): Acute psychosis following the use of topical ciprofloxacin. Arch Ophthalmol; 120(5):665-666.

Tsien RY (1989): Fluorescent probes of cell signaling. Ann Rev Neurosci; 12:227-253.

Valdeolmillos M, Nadal A, Contreras D, Soria B (1992): The relationship between glucose-induced K^+ATP channel closure and the rise in [Ca2+]i in single mouse pancreatic beta-cells. J Physiol; 455:173-186.

Vallurupalli S, Huesmann G, Gregory J, Jakoby MG 4th (2008): Levofloxacin-associated hypoglycaemia complicated by pontine myelinolysis and quadriplegia. Diabet Med; 25(7):856-859.

Van Bambeke F, Tulkens PM (2009): Safety profile of the respiratory fluoroquinolone moxifloxacin: comparison with other fluoroquinolones and other antibacterial classes. Drug Safety; 32(5):359-378.

Van de Casteele M, Kefas BA, Cai Y, Heimberg H, Scott DK, Henquin JC, Pipeleers D, Jonas JC (2003): Prolonged culture in low glucose induces apoptosis of rat pancreatic β-cells through induction of c-myc. Biochem Biophys Res Commun; 312(4):937-944.

Van den Bergh V, Boens N, De Schryver FC, Ameloot M, Steels P, Gallay J, Vincent M, Kowalczyk A (1995): Photophysics of the Fluorescent Ca^{2+} Indicator Fura-2. Biophys J; 68(3):1110-1119.

van Der Linden PD, Stricker BHCh, Leufkens HGM, Herings RMC, Nab HW, Simonian S (2001): Fluoroquinolone use and the change in incidence of tendon ruptures in the Netherlands. Pharm World Sci; 23 (3):89-92.

Vancutsem PM, Schwark WS (**1992)**: Effects of fluoroquinolone antimicrobials alone and in conjunction with theophylline on seizures in amygdaloid kindled rats: mechanistic and pharmacokinetic study. Epilepsy Res; 13:59-71.

Varadi A, Ainscow EK, Allan VJ, Rutter GA (2002): Involvement of conventional kinesin in glucose-stimulated secretory granule movements and exocytosis in clonal pancreatic beta-cells. J Cell Sci; 115(Pt 21):4177-4189.

Velissariou M (2006): The use of fluoroquinolones in children: recent advances. Expert Rev Anti Infect Ther; 4 (5):853-860.

Vetterlein F, Senske D, Bornkessel C, Schmidt G (1985): Effects of tolbutamide on blood flow in islets and exocrine tissue of the rat pancreas. Eur J Pharmacol; 113(3):395-398.

Vial T, Chauplannaz G, Brunel P, Leriche B, Evreux JC (1995): Exacerbation of myasthenia gravis by pefloxacin. Rev Neurol (Paris); 151 (4):286-287.

Vick-Fragoso R, Hernández-Oliva G, Cruz-Alcázar J, Amábile-Cuevas CF, Arvis P, Reimnitz P, Bogner JR; STIC Study Group (2009): Efficacy and safety of sequential intravenous/oral moxifloxacin vs intravenous/oral amoxicillin/clavulanate for complicated skin and skin structure infections. Infection; 37(5):407-417.

Vorndran C, Minta A, Poenie M (1995): New fluorescent calcium indicators designed for cytosolic retention or measuring calcium near membranes. Biophys J**;** 69 (5):2112-2124.

Wacke R, Förster S, Adam U, Mundkowski RG, Klar E, Hopt UT, Drewelow B (2006): Penetration of moxifloxacin into the human pancreas following a single intravenous or oral dose. J Antimicrob Chemother; 8(5):994-999.

Wagnières GA, Star WM, Wilson BC (1998): In vivo fluorescence spectroscopy and imaging for oncological applications. Photochem Photobiol; 68(5):603-632.

Walters JD, Nakkula RJ (2003): Ciprofloxacin transport by chemoattractant-activated polymorphonuclear leukocytes: Regulation by priming and protein kinase C. Antimicrob Agents Chemother; 47(10):3345-3348.

Whiteley MS, Worlding J, Patel S, Gibbs KB (1993): Hypoglycemia in a diabetic patient, associated with ciprofloxacin therapy. Pract Diabetes; 10:35.

WHO 2005 Guidelines for the control of shigellosis including epidemics due to Shigella dysenteriae type 1 avaible at URL:http://whqlibdoc.who.int/publications/2005/9241592330.pdf [Accessed 08.06.2010].

Williams D, Hopkins S (1998): Safety of trovafloxacin in treatment of lower respiratory tract infections. Eur J Clin Microbiol Infect Dis; 17:454-458.

Winzell MS, Ahrén B (2007): Role of VIP and PACAP in islet function. Peptides; 28(9):1805-1813.

Wolf DE (2007): Fundamentals of florescence and fluorescence microscopy in digital microscopy third edition; Academic press: p 69.

Wolters GH, Vos-Scheperkeuter GH, van Deijnen JH, van Schilfgaarde R (1992): An analysis of the role of collagenase and protease in the enzymatic dissociation of the rat pancreas for islet isolation. Diabetologia; 35(8):735-742.

Yamada C, Nagashima K, Takahashi A, Ueno H, Kawasaki Y, Yamada Y, Seino Y, Inagaki N (2006): Gatifloxacin acutely stimulates insulin secretion and chronically suppresses insulin biosynthesis. Eur J Pharmacol; 553:67–72.

Yamamoto T, Ricordi C, Messinger S, Sakuma Y, Miki A, Rodriguez R, Haertter R, Khan A, Alejandro R, Ichii H (2007): Deterioration and variability of highly purified collagenase blends used in clinical islet isolation. Transplantation; 84(8):997-1002.

Yang SN, Berggren PO (2005): B-cell CaV channel regulation in physiology and pathophysiology. Am J Physiol- Endocrinol Metab; 288(1):E16-28.

Yang SN, Berggren PO (2006): The role of voltage-gated calcium channels in pancreatic β-cell physiology and pathophysiology. Endocr Rev; 27(6):621-676.

Yap YG, Camm AJ (2003): Drug induced QT prolongation and torsades de pointes. Heart; 89(11):1363-1372.

Yip C, Lee AJ (2006): Gatifloxacin-induced hyperglycemia: a case report and summary of the current literature. Clin Ther; 28(11):1857-1866.

Zaitsev SV, Efendić S, Arkhammar P, Bertorello AM, Berggren PO (1995): Dissociation between changes in cytoplasmic free Ca^{2+} concentration and insulin secretion as evidenced from measurements in mouse single pancreatic islets. Proc Natl Acad Sci (USA); 92(21):9712-9716.

Zawalich WS, Yamazaki H, Zawalich KC, Cline G (2004): Comparative effects of amino acids and glucose on insulin secretion from isolated rat or mouse islets. J Endocrinol; 183(2):309-319.

Zeltser D, Justo D, Halkin A, Prokhorov V, Heller K, Viskin S (2003): Torsades de pointes due to non cardiac drugs: most patients have easily identifiable risk factors. Medicine (Baltimore); 82:282-290.

Zerangue N, Schwappach B, Jan YN, Jan LY (1999): A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K_{ATP} channels. Neuron; 22:537-548.

Zhanel GG, Ennis K, Vercaigne L, Walkty A, Gin AS, Embil J, Smith H, Hoban DJ (2002): A critical review of the fluoroquinolones: focus on respiratory infections. Drugs; 62 (1):13-59.

Zhang L, Ging NC, Komoda T, Hanada T, Suzuki T, Watanabe K (2005): Antibiotic susceptibility of mammalian mitochondrial translation. FEBS Lett; 579(28):6423-6427.

Zhang L, Wei MJ, Zhao CY, Qi HM (2008): Determination of the inhibitory potential of 6 fluoroquinolones on CYP1A2 and CYP2C9 in human liver microsomes. Acta Pharmacol Sin; 29(12):1507-1514.

Zhao B, Chignell CF, Rammal M, Smith F, Hamilton MG, Andley UP, Roberts JE (2010): Detection and Prevention of Ocular Phototoxicity of Ciprofloxacin and Other Fluoroquinolone Antibiotics. Photochem Photobiol; 86:798-805

Zimny S, Gogolin F, Abel J, Gleichmann H (1993): Metallothionein in isolated pancreatic islets of mice: induction by zinc and streptozotocin, a naturally occurring diabetogen. Arch Toxicol; 67:61-65.

Zimpfer A, Propst A, Mikuz G, Vogel W, Terracciano L, Stadlmann S (2004): Ciprofloxacin-induced acute liver injury: case report and review of literature. Virchows Arch; 444(1):87-89.

Zünkler BJ, Claassen S, Wos-Maganga M, Rustenbeck I, Holzgrabe U (2006): Effects of fluoroquinolones on HERG channels and on pancreatic β-cell ATP-sensitive K^+ channels. Toxicology; 228(2-3):239-248.

Zvonar R (2006): Gatifloxacin-induced dysglycemia. Am. J Health Syst Pharm; 63(21):2087-2092.